Chapter 1

Introduction

1.1 Orientation to the study

The goal of radiotherapy is to deliver a sufficiently high dose of radiation in order to sterilise tumour cells, with minimal damage to surrounding normal tissues.[1] The fact that the interaction of radiation with cells, tissues and organs is a non-specific random process, with no specificity for tumour cells, makes the goal not easily achievable because of the presence of surrounding normal tissues. For this reason, with conformal radiation therapy treatment techniques, a dose fraction is usually delivered using multiple treatment fields to conform the dose to the planning target volume in order to minimise the dose to surrounding normal tissues.

Since the year 1990, an intense effort has been made in conformal radiotherapy treatment planning to ensure that the prescribed physical doses are accurately, uniformly and reproducibly delivered throughout the entire treatment course.[2] However, with the advent of radiotherapy techniques such as Intensity Modulated Radiotherapy (IMRT), which utilises long treatment times (15 to 45 minutes), the possible impact of temporal effects such as fraction treatment time and the sequencing of treatment fields on treatment outcome has now come to the fore.[2]

The biological impact of fraction treatment time on cell survival has been thoroughly studied and the possible biologic impact of beam sequencing has also been acknowledged. However, the studies outlining the optimal treatment field sequence that yields improved treatment outcomes are still in their infancy.[2, 3] These studies have, indicated that tumour types with low α/β ratios respond better to the manipulation of treatment field sequence than tumour types with high α/β ratios. These studies[2, 3] also determined that when higher dose fields are treated in the beginning of the fraction, a higher cell survival is observed than when lower dose fields are treated in the beginning of the treatment fraction. The current study was aimed at further investigating the optimal treatment field sequence when treating tumour types with low α/β ratios, in particular prostate tumours, using conformal radiotherapy treatment techniques.

1.2 Background and rationale

A typical clinical conformal radiotherapy treatment begins with a Computed Tomography (CT) of the body region of interest. The CT is then imported to a computer-based radiotherapy treatment planning system, where the clinical target volume and normal tissue structures are delineated. For three-dimensional (3-D) conformal radiotherapy, computerised algorithms generally create a treatment plan using a technique called “forward planning” where the beam sizes, weights and positions are specified by a

dosimetrist and dose distributions are then calculated. The treatment plan is created according to the total dose, dose per fraction and total treatment time prescribed by the radiation oncologist for the particular tumour type and tumour stage. The resulting treatment plan should satisfy the *International Commission of Radiation Units' and Measurement (ICRU) 50 criteria of an acceptable plan*.[4]

At the treatment unit, the prescribed daily dose is delivered using multiple beams from different directions. At present, no guidelines exist as to how these multiple beams should be sequenced during treatment.[2] As a result, the order in which the treatment fields are sequenced is chosen by an individual radiation therapist delivering the treatment on that day and is not based on any pre-determined criteria. This random sequencing of beams was observed by the researcher at the Radiation Oncology Department of Steve Biko Academic Hospital.

This observation invoked a question as to whether a significant radiobiological effect can be induced by these different treatment field sequences. A search was conducted through the University of Pretoria information specialists, using the Medline (Ovid) database, the electronic database Pubmed and keywords (temporal optimisation, beam sequencing, order of dose application, and dose optimisation).

Both experimental and mathematically modelled radiobiological studies revealed that the degree of both tumour and normal tissue cell kill may be dependent on the temporal pattern (overall fraction time and pattern of dose delivery) of dose delivery during treatment.[2, 3, 5, 6, 7, 8, 9] The impact of overall fraction time on cell kill has been thoroughly studied.[10, 11, 12, 13, 14, 15, 16, 17] These studies analysed the effect of overall fraction time on cell kill and concluded that cell kill can be increased by keeping overall fraction time to a minimum. The impact of the pattern of applied dose has also been studied, but most studies failed to analyse how the treatment field sequence, i.e. the pattern of dose delivery, can be manipulated to optimise cell kill. Only three published studies, an *in vitro* study by Murphy and Lin[3], and modelled studies by Brenner, Hall, Huang & Sachs[18] and Altman & Chmura[19], that attempted to determine how treatment field sequence can be manipulated to influence cell kill could be found by the researcher, using the above-mentioned databases and keywords.

Altman *et al*'s mathematically modelled study[19] led to the development of two dose delivery patterns: 1) the triangular and 2) the V-shaped pattern of dose delivery. Altman *et al* investigated these dose-delivery patterns when applied to simplified IMRT clinical cases. IMRT differs from conformal radiation therapy technique in that it utilises a large number of external radiation fields that feature a high degree of spatial and fluence modulation for each beam.[19] Conformal radiation therapy, on the other hand, uses a

small number of fields without fluence modulation. Hence, conformal radiation therapy takes about two to five minutes to deliver a treatment fraction, whereas IMRT requires 15 to 45 minutes to deliver the same fractional dose.[16]

Altman *et al*'s IMRT study[19] concluded that temporal optimisation can be achieved by using triangular and V-shaped patterns of dose delivery to take advantage of time-dose effects (increase cell kill in tumours and decrease cell kill in normal tissues) within treatments, not only for IMRT but for non-IMRT radiotherapy techniques which use multiple radiation fields of differing doses as well.[19] Altman *et al*'s suggestion of implementing the dose-delivery patterns in non-IMRT radiotherapy techniques led to the undertaking of the current study.

1.3 Statement of the research problem

The currently used patterns of dose delivery at Steve Biko Academic Hospital's Radiation Oncology Department are non-standardised. The pattern of dose delivery is rather determined by each individual radiation therapist, without coherence between different sessions of treatment. The lack of guidelines on how multiple fields should be sequenced during treatments leads to this haphazard beam sequencing and this anomaly is not inherent in the local setting only, but is also observed in international external beam radiation therapy practice worldwide.[2] The development of triangular and

V-shaped patterns of dose delivery by Altman *et al*[19], brought to light the fact that the pattern of dose delivery can be manipulated and possibly standardised in daily treatments in order to increase tumour cell kill, particularly in tumour types with low α/β ratios.

No study (experimental or modelled) could be found, using the above-mentioned search strategies, that applies these newly developed patterns of dose delivery to the more often used conformal radiotherapy treatment techniques.

1.4 Aim of the study

The main aim of the study was to compare tumour cell radiosensitivity, when using the triangular, the V-shaped or the conventional pattern of dose delivery, in tumour types with low α/β ratios in 3-D conformal radiation treatment techniques.

The sub-aims of the study were to:

- Compare triangular and V-shaped patterns of dose delivery on prostate tumour cells with α/β ratios of 1 Gy, 1.5 Gy and 3 Gy;
- Compare triangular and conventional pattern of dose delivery on tumour types with α/β ratios of 1 Gy, 1.5 Gy and 3 Gy;

- Compare V-shaped and conventional patterns of dose delivery on tumour types with α/β ratios of 1 Gy, 1.5 Gy and 3 Gy.

1.5 Hypotheses

Null hypothesis$_1$: There is no significant difference in tumour cell radiosensitivity between the triangular and V-shaped patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

Null hypothesis$_2$: There is no significant difference in tumour cell radiosensitivity between the triangular and conventional patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

Null hypothesis$_3$: There is no significant difference in tumour cell radiosensitivity between the V-shaped and conventional patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

Alternative hypothesis$_1$: The triangular pattern of dose delivery will result in significantly lower cell radiosensitivity than the V-shaped pattern of dose delivery when applied to

prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

Alternative hypothesis$_2$: The triangular pattern of dose delivery will result in significantly lower cell radiosensitivity than the conventional patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

Alternative hypothesis$_3$: The conventional patterns of dose delivery will result in significantly lower cell radiosensitivity than the V-shaped pattern of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques.

1.6 Significance of the study

The success of a course of radiotherapy treatment is judged by increased tumour control and reduced normal tissue complications. Increased tumour control is dependent on increased tumour cell kill. Tumour cell kill is dependent on the optimisation of both spatial and temporal factors within daily radiotherapy treatments. Spatial factors have been thoroughly studied and have been optimised over the years in an attempt to increase tumour cell kill. Temporal factors, on the other hand, have been ignored because of the assumption that biological effect is solely dependent on the size

of the dose and the overall treatment time.[2] The study explored the impact of temporal factors, in particular the dose-delivery pattern, in daily treatments with the aim of optimising them. The result of the study thus serves to ascertain the current practice of beam sequencing. Contrary results have been observed in IMRT studies.[19]

1.7 Definitions of key variables

In this section, conceptual definitions of variables are identified. A conceptual definition provides a variable with theoretical meaning and is either derived from literature or through general usage of the concept.[20]

Triangular pattern of dose delivery

The triangular pattern of dose delivery concerns the delivery of the dose of radiation that begins the fraction with a low-dose (less weighted) field at the beginning of the fraction, followed by high-dose (more weighted) fields in the middle of the fraction, and the remaining low-dose fields at the end of the fraction, as illustrated in Figure 1.1.

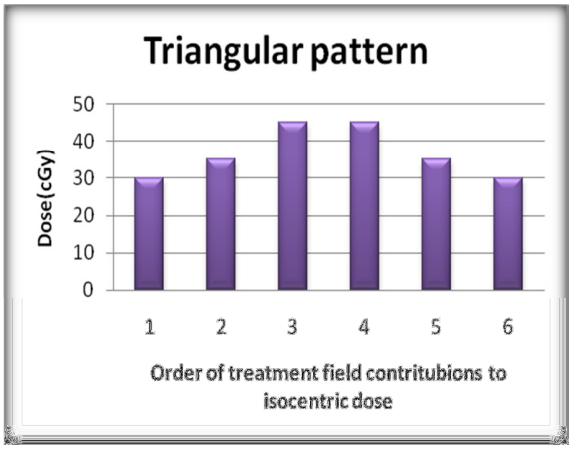

Figure 1.1: Bar graph of the triangular pattern of dose delivery

V-shaped pattern of dose delivery

The V-shaped pattern of dose delivery is the delivery of the dose of radiation that begins the fraction with a high-dose field at the beginning of the fraction, followed by low-dose fields in the middle of the fraction, and high-dose fields at the end of the fraction, as illustrated in Figure 1.2.

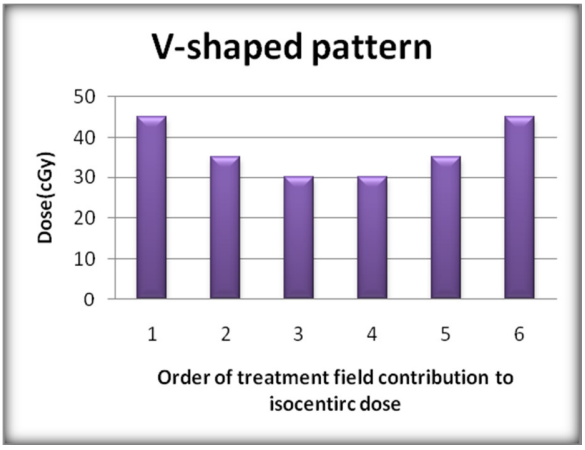

Figure 1.2: Bar graph of the V-shaped pattern of dose delivery

Conventional pattern of dose delivery

A conventional pattern of dose delivery is the pattern of dose delivery that is used most often at the Radiation Oncology Department of Steve Biko Academic Hospital to deliver a prescribed fractional dose. Several conventional patterns are used in clinical practice. The pattern selected for this study is the one used by most radiation therapists in the radiation oncology department of the mentioned setting. The conventional patterns are not standardised, but are based on the decision of the individual radiation therapist and are mainly selected for convenience. The conventional pattern adopted for the study is shown in Figure 1.3.

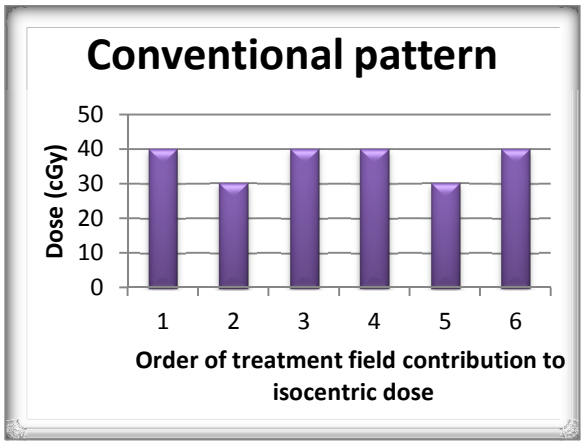

Figure 1.3: Diagrammatic presentation of the conventional pattern used in the study

Tumour cell survival

In radiation biology, a cell has survived when it has retained its reproductive capability after a dose of radiation. A cell that is still physically alive but has lost its reproductive capability is deemed dead.

Cell Radiosensitivity

The sensitivity of cells to damage by radiation, expressed by the mean inactivation dose (MID)

1.8 Outline of the dissertation

From Chapter 1, it emerged that the pattern of dose delivery, during the course of radiation therapy, may be adapted to influence the radiosensitivity of cells. In Chapter 2 theoretical background, based on a literature review, is presented to provide a conceptual framework for the rationale behind the suggested dose-delivery patterns. The research methodology adopted for the study to investigate the problem and to achieve the aim of the study is described in Chapter 3. Chapters 4 and 5 give a presentation and discussion of the findings of the study; and in Chapter 6, the main conclusions of the study are summarised and recommendations for practice and for further research made. A flow diagram presenting the structure of the dissertation is shown in Figure1.4.

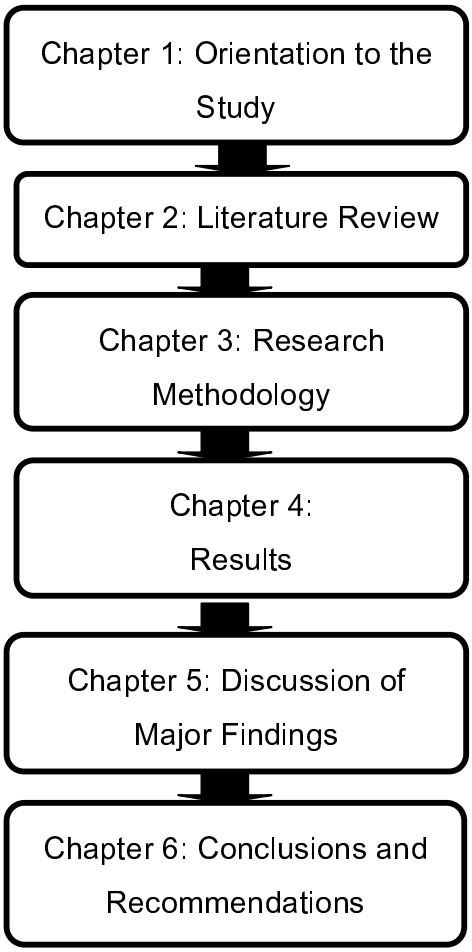

Figure 1.4: Flow diagram of the dissertation structure

1.9 Conclusion

This chapter provided a brief overview of the problem investigated in the study, linking what is already known about the problem to what the study aimed to investigate. The research problem was identified and reasons for conducting the study were given. The anticipated positive contributions of the results of the current study towards existing practice were highlighted.

Chapter 2

The temporal pattern of radiation dose delivery in conformal radiotherapy treatment techniques

2.1 Introduction

Critical to the understanding of this study is the fact that it is a mathematically modelled investigation based on the radiation biology of an entire course of radiation treatment. In radiation biology, studies are conducted in different fields: laboratory (*in vitro*) studies, mathematically modelled studies, and studies in application in clinical practice.[21] Modelled studies have thus far been an important means of explaining the link between what is observed in the laboratory, and what is observed and implemented in clinical practice, as shown in Figure 2.1.

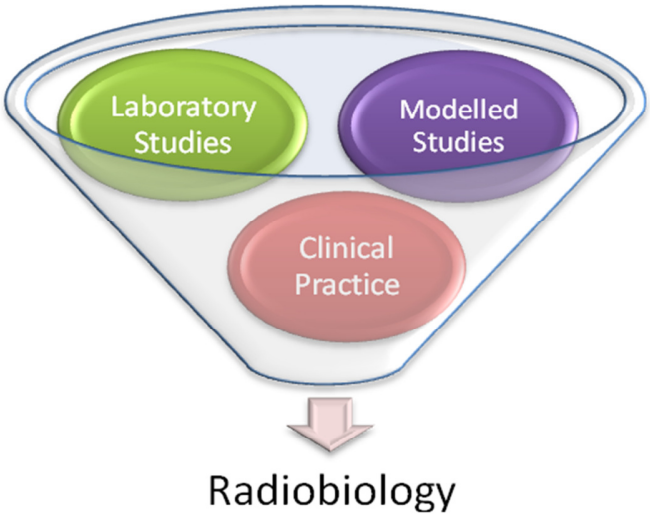

Figure 2.1: Diagram of the relation of different fields of study in radiation biology

2.2 Overview of literature review

As previously stated, radiation therapy is aimed at eradicating tumour cells, while sparing normal tissues.[1] It is common practice to deliver radiation treatments in 2 Gy fractions, five fractions a week, up to 33 to 39 fractions in seven to eight weeks to achieve this aim.[3] Each daily dose is usually delivered using multiple treatment beams, each of which contribute differing doses to the isocentric dose. In a single treatment fraction and consequently in the entire treatment course, the desired endpoint of optimal tumour cell kill is dependent on a number of factors, including:

- Biologic factors (radiation capacity of the cells, intra-tumour inhomogeneities, oxygen content);
- Spatial factors (radiation beam directions, field weight, treatment field size, dose distribution);
- Temporal factors (fraction duration, dose-delivery pattern);
- Dose rate; and
- Type of radiation.[2, 3, 21]

The focus of the current study concerns temporal factors, in particular the dose-delivery pattern, as illustrated in Figure 2.2.

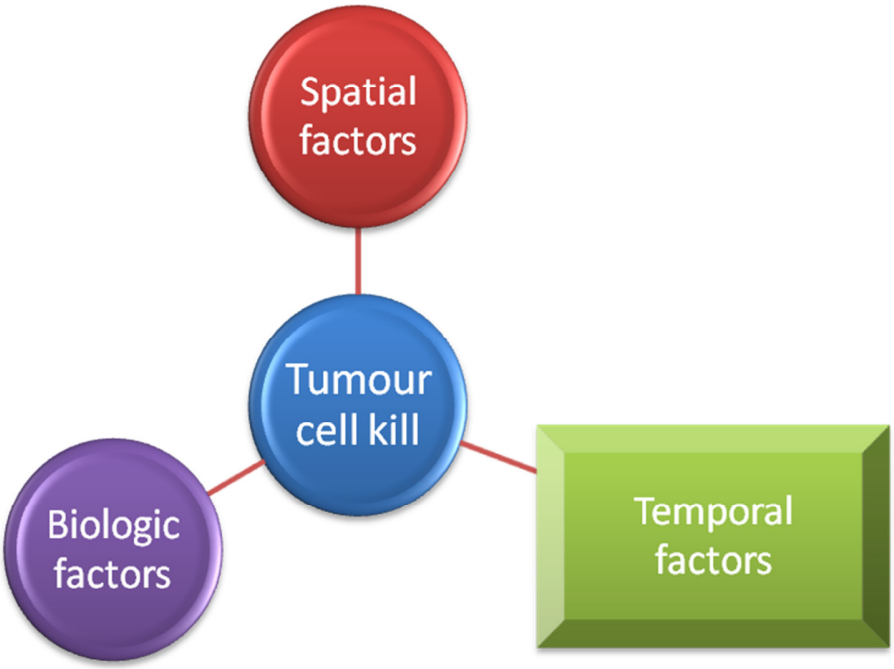

Figure 2.2: Factors affecting tumour cell kill in each fraction of radiation

2.3 Temporal factors in conformal radiotherapy

For external beam radiotherapy, three significant time-related factors that influence the response of tissues to irradiation exist: 1) the daily fractionation schedule, 2) the duration of each individual fraction, and 3) the temporal pattern for delivering individual radiation fields.[3, 22] In each fraction of radiation, two of the time-related factors viz. fraction duration and temporal pattern of dose delivery influence the radiobiological effects in that fraction.

Theoretical and experimental studies[2, 18, 19] indicate that the temporal pattern (fraction duration and intra-fraction sequence for delivering individual radiation fields) of cell

irradiation influences the competition between surviving tumour cell proliferation, cell kill and sub-lethal damage repair. This competition, in turn, influences overall cell survival and hence tumour control.

The impact of temporal effects in radiation therapy has been investigated in a number of studies.[10, 11, 12, 13, 14, 15, 16, 17] In these studies, the focus has been on the interaction of longer treatment times and cell repair, which is mostly associated with IMRT. Less attention has been paid to the intra-fraction dose-delivery pattern for individual radiation fields. These temporal factors are discussed below.

2.3.1 Fraction duration

Fraction duration is the total time measured from the time that the first beam is turned "on" until the last beam is turned "off". The impact of fraction duration on cell survival has long been acknowledged. In a classic study, Elkind and Sutton (1959)[23] performed an *in vitro* experiment to investigate the impact of fraction duration on cell survival. A Chinese hamster cell line was irradiated with 992 cGy acutely, and in the next phase of the experiment the same cell line was irradiated with 505 cGy and 487 cGy over an interval of 30 to 40 minutes, separated by 12 hours. The results of the experiment indicated a 0.19 per cent survival for the acute irradiation and a 0.78 per cent survival when the two doses were separated by 12 hours.[23]

Berry and Oliver (1964)[24] also investigated this phenomenon in human cells. They exposed He La cells to a single fraction of 4 Gy and to two fractions of 2 Gy each separated by one to two hours. When compared to a 4 Gy single fraction, cell survival was increased by a factor of 1.15 for a one-hour separation and by a factor of 1.4 for the two-hour separation.[24]

More recent studies [10, 16, 22, 25, 26, 27, 28, 29, 30] analysed these effects on cell survival as they relate to modern therapeutic techniques, and show that there is a reciprocal relationship between cell kill and fraction duration. In particular, Sterzing, Munter and Schafer[25] conducted an *in vitro* study to explore the impact of fraction time on cell survival. Two human cell lines, lymphoidblastic cells and melanoma cells, were irradiated with 2 Gy over times ranging from five to 30 minutes. Both cell lines showed a statistically significant increase in cell survival as the treatment time increased. Similar results are echoed in other studies.[5, 17, 20, 21, 37, 31, 32] These observations prompted recommendations to adjust treatment planning and delivery processes to compensate for the protracted fraction dose-delivery times that are characteristic of IMRT, hypofractionated radiotherapy and radiosurgery.

With increasing evidence of this dose prolongation effect, the question then regards the biology underlying these studies. A comprehensive analysis of cellular mechanisms which give rise to this effect can be found in literature.[31, 32] Briefly, lethal damage of cells caused by low Linear Energy Transfer (LET) radiation is the cumulative result of multiple independent radiation damage events rather than one catastrophic event. With the increasing protraction of fraction duration, a longer time period exists for adequate repair of deoxyribonucleic acid (DNA) damage during the fraction.

2.3.2 Dose-delivery pattern

Another variable that affects the degree of cell kill is the functional form of how the dose is delivered over time in each fraction. Stewart and Traub[5] from the Pacific Northwest National Laboratory (Portugal), in their effort to determine how the temporal pattern of dose delivery can be used to exploit the full potential of radiotherapy, developed software to estimate tumour control probabilities (TCPs) using tissue constructs. They used the Monte Carlo N-particle (MCNP) Monte Carlo code to compute the absorbed dose distribution for a cylindrical tissue construct that was irradiated by a broad parallel mega electron volt (MeV) beam from eight different directions. Given the dose distributions, a computer application, VOXEL, was then used to construct the absorbed dose rate function and to describe the sequence of radiation pulses experienced by the cells in each voxel. The computer application VOXEL uses the LPL radiobiological

model to estimate the fraction of the initial number of cells that survive in each tissue region. The authors' conclusion suggests that the biologic optimisation of the way in which dose fractions are delivered (beam sequence and intensities) could improve the efficiency of some radiation treatments by factors in the order of five to 20 per cent (iso-effect dose).[5] The study could not, however, determine how the beam sequence and beam intensities could be re-arranged to improve radiation treatments by the mentioned five to 20 per cent.

In a similar effort, Brenner *et al*[18] used a computer-based numerical optimisation by adjusting doses in different parts of a single fraction, keeping the dose per fraction and time per fraction fixed. The study used the linear quadratic formalism, Normal Tissue Complication Probability (NTCP) model and TCP model to determine mathematically a protocol of radiation delivery which would exploit the therapeutic difference between early responding tissues, such as tumours, and late responding tissues. Four protocols were analysed: in Protocol A, a continuous low dose rate was used throughout; in Protocol B a high dose was delivered at the beginning of the fraction, followed by a continuous low dose rate in the middle of the same fraction and another high dose at the end of the fraction; in Protocol C a high dose was given followed by a delay, then a continuous low dose followed by another delay and another high dose at the end of the

fraction; in Protocol D multiple acute doses were given, with the first and the last fraction being the largest.

Protocol B with high doses at the beginning and end of treatment had the most sparing of both tumour and normal tissue. The reason for the sparing is that damage inflicted at the beginning and end of treatment has less chance to interact with damage caused by doses from the previous fraction and from the subsequent fraction. Brenner *et al* concluded that Protocol B had the most potential for general application. The study's emphasis was, however, based on brachytherapy and other accelerated radiotherapeutic protocols, but not on external beam and conventional protocols.[18]

Lin *et al* [2] conducted an *in vitro* study to test whether the commonly prescribed daily dose of 2 Gy produces the same biological effects when delivered as "partial fractions" of different sequences. In their study, 11 human and animal cell lines were irradiated with 2 Gy divided into two or more partial fractions similar to the delivery sequence used in clinical practice. Lin *et al* managed to show that when a dose of 2 Gy is delivered in a sequence of two small doses (smaller than 0.5 Gy) followed by large doses (greater than 0.5 Gy), cell survival is significantly (10 to 20 per cent) smaller than when the same dose is delivered in a pattern with large doses (greater than 0.5 Gy) followed by small doses (smaller than 0.5 Gy). These differences were attributed to two mechanisms: (1)

low-dose hypersensitivity at doses of below 0.5 Gy; and (2) increased radioresistance at doses of above 0.5 Gy. They concluded that because of these mechanisms, it is possible that the biological effect of 2 Gy delivered clinically may be dependent on the sequence in which the treatment fields are ordered. They suggested a general pattern of sequencing the treatment fields in such a way that the low dose fields (smaller than 0.5 Gy) are treated first, followed by the high dose fields (greater than 0.5 Gy). Their study was, however, limited to IMRT and radiosurgery treatment techniques which use a large number of fields and where treatment fields that deliver doses that are larger than 0.5 Gy per field are applicable.[2]

Altman *et al* [19] conducted a modelled study to determine if and how a set of doses could be arranged to maximise or minimise cell kill by using a defined array of dose values and specific fraction duration. The order for the dose values was randomly selected and a surviving fraction of cells for each order was calculated using a Monte Carlo simulation and linear quadratic formalism. The process was repeated for all permutations of dose order (field order) using the same set of input values. Each time, the surviving fraction of cells was calculated and the dose order that generated it was recorded. Ten IMRT treatment plans, using six to nine treatment fields, were used. Altman *et al* concluded that the temporal pattern of dose delivery can be arranged to maximise cell kill by forming a triangular pattern of dose, with the smallest doses

applied during the beginning and end of irradiation, and the largest dose applied in the middle of the fraction. Minimisation of cell kill can also be achieved by forming a V–shaped dose-delivery pattern, with the largest doses applied at the beginning and end of the fraction, and the smallest doses in the middle of the fraction. Their analysis also demonstrated that the difference in cell survival generated by the rearrangement of pattern of applied dose increases with increasing fraction duration and dose per fraction, and decreases with increasing tissue α/β ratios, as illustrated in Table 2.1.[19]

Table 2.1: A summary of the mean percentage difference values calculated by α/β ratio and fraction duration[19]

Tissue α/β ratios	Fraction Duration (min)	Mean difference in cell kill (%)
3	20	3
	30	3.7
	40	4.2
10	20	0.9
	30	1.1
	40	1.3

The results of the study by Altman *et al* were validated *in vitro*; two cell lines, one with a low α/β ratio (3 Gy) and one with a high α/β ratio (10 Gy), were plated and irradiated with 9 Gy in triangular and V-shaped patterns with a delivery time of 20 minutes each.

The triangular and the V-shaped patterns showed a relative cell survival difference of 15.2 per cent to 18.6 per cent when applied to a low α/β ratio cell line (PC-3), as opposed to a 4.5 per cent relative cell survival difference when applied to a high α/β ratio cell line (SQ-20b). These *in vitro* results, therefore, validated the assertions that the specific pattern of dose application, either triangular or V-shaped, does influence the amount of cell kill.[32] "These results imply that the order of the temporal pattern of applied dose could have clinically significant implications, and that methods of optimizing the temporal pattern of dose could be used to directly affect treatment outcome."[19]

Altman *et al*'s observations that, the effects of the pattern of dose delivery were more evident as the α/β ratio decreased led to prostate tumour, which has an α/β ratio in the range of 1 to 3 Gy,[33] to be adopted for use in the current study in order to demonstrate any difference in cell kill from conformal radiotherapy. It should be noted that Altman *et al*'s study used longer fraction delivery times that are consistent with the times used in IMRT, whereas short treatment times consistent with those used in conformal radiotherapy were simulated in the current study. Another difference between the Altman *et al* study and the current study is that Altman *et al* used α/β values of 3 Gy and 10 Gy, while the current study only used low α/β values of 1 Gy, 1.5 Gy and 3 Gy.

2.4 Biological motivation for the suggested triangular and V-shaped patterns of dose delivery

The triangular and V-shaped patterns arise from an analysis of cell kill within a differential time element of dose delivery. From the linear quadratic model, radiation damage has three components: (1) creation of lethal single tract lesions (alpha term in linear quadratic equation); (2) creation of sub-lethal lesions which will interact with sub-lethal lesions that are created later during the same fraction to form lethal lesions (beta term in linear quadratic equation); and (3) creation of sub-lethal lesions which interact with sub-lethal lesions formed during a previous fraction, to form lethal lesions.[19] For clarity, these damage types will be referred to in this study as type-1, type-2 and type-3 damage, respectively. Type-1 damage, being single track lesion, is only dependent on the dose per fraction given and does not have a temporal component, while type-2 and type-3 damage each influence temporal effects.[19]

At the beginning of a fraction, type-2 damage dominates. However, with increasing time in a fraction, the prominence of type-2 damage decreases. This is due to both the repair of sub-lethal damage sites and diminishing opportunity for future radiation damage before the end of the fraction. At the beginning of the fraction there is little or no type-3 damage. The amount of type-3 damage increases with time within a fraction, becoming the dominant form of damage at the end of the fraction. This is because of the increase in sub-lethal damage sites with which other damaged sites can interact. Therefore, the

dose given near the beginning of a fraction is less lethal owing to a lack of type-3 cell kill, while the dose given near the end of a fraction results in reduced lethality due to a deficiency in type-2 cell kill. Hence, reduced cell kill is achieved through the concentration of more dose near the ends of a fraction, as is achieved when using a V-shaped pattern of dose delivery. The opposite arrangement (triangular pattern) results in increased cell kill.[19, 32]

2.5 Biological modelling of temporal effects

Temporal effects relating to radiation therapy are generally analysed in terms of cell survival, using the linear quadratic model.[34] The model was originally developed from the perspective of sub-cellular bio-physical events; but, in clinical practice, the model also provides a satisfactory and useful explanation of fractionation and dose rate effects observed at a macroscopic level. The model may be applied to both tumour and normal tissues and is, therefore, particularly useful in evaluating the impact of altered patterns of radiation dose delivery on the overall therapeutic index.[34] The linear quadratic (LQ) model exists in various forms, but all forms are based on the assumption that the overall biological effect of radiation is reflected in the surviving fraction of cells.

The LQ model expresses cell survival as:

$$S = \exp(-\alpha D - G(t)\,\beta D^2) \qquad (2.1)$$

where S is the fraction of cells surviving after a dose per fraction D, and α and β relate to the tissue's ability to repair radiation damage. The factor G (t) is the dose protraction factor.[23] The simple way to conceptualise the LQ model is that the α term corresponds to cell death from radiation damage which is irreparable or lethal, while the β term corresponds to cell death from radiation damage which is repairable or sub-lethal. Cell kill due to radiation thus results from lethal damage or as a result of the interaction between sub-lethal damages to form lethal damage. Clearly, if some time elapses between the first sub-lethal damage and the second sub-lethal damage, there exists a possibility that the first damage may be repaired before interacting with the second one. If the first damage is repaired, the second damage will not create lethal damage. This repair that occurs between the first and second damage is accounted for by the dose protraction factor G (t). This factor theoretically tends to one when the dose rate is very high and repair is negligible during irradiation. The factor G (t) contains the temporal variables of the fractional dose.[35]

The existence of the dose protraction factor in the LQ model lays the foundation for the arguments of the current study that the temporal pattern of dose delivery could influence cell kill through sub-lethal damage repair. The arguments emanate from the model itself.

Other models that use systems of ordinary differential equations to link the formation and repair of double-strand breaks (DSBs) to cell killing have also been proposed. The two lesions kinetic model (TLK) and the repair-misrepair model (RMR) are two outstanding examples of such models. In these models, the phenomenon of DSBs rejoining is modelled using first order (linear) and second order (non-linear) repair mechanisms. Several authors[9, 30, 36, 37] have shown that these two models can be used to derive the standard LQ formula when using small doses and dose rates because the LQ model does not give accurate results when used at low doses and dose rates.

The TLK model has been argued to better link the biochemical processing of the DSBs and cell killing than the LPL, RMR and linear quadratic models.[36] Although the LPL, RMR and LQ models have been argued by Stewart [38] to be unsatisfactory in linking biochemical processing of DSBs to cell killing, during the test simulations conducted to validate the models in the Virtual Cell (VC) programme, the LPL model yielded results that approximated those observed *in vitro*, and was thus adopted for use in the current study. The model is discussed in Section 2.5.1.

2.5.1 Lethally Potentially Lethal (LPL) model

The original LPL model assumed two general classes of DNA damage, potentially lethal lesions and lethal lesions. Potentially lethal lesions are not lethal unless they interact with other potentially lethal lesions through pairwise interaction processes. Some potentially lethal lesions are repaired correctly or they are misrepaired in a way that is not lethal to the cell. A lethal lesion, on the other hand, is unrepairable damage that prevents a cell from dividing.[36] The most commonly used version of the LPL model assumes that a potentially lethal damage is the same as a DSB, and is represented by the equation:

$$\frac{d\bar{L}_{dsb}(t)}{dt} = 2D(t)Y\sum_{dsb} - \{\lambda_{dsb} + \eta_{dsb}\bar{L}_{dsb}(t)\}\bar{L}_{dsb}(t) \qquad (2.2)$$

Where $\bar{L}$ (t) is the expected average number of DSBs in a cell at time *t, D(t)* is the instantaneous absorbed dose rate at time t, Y is the number of base pairs per cell, $\sum dsb$ is the expected number of DSBs initially created by radiation per nucleotide per gray, λ_{dsb} is the DSB repair probability and η_{dsb} is the DSB-DSB interaction probability.[36]

The LPL model has been criticised for not being able to account for the creation of non-lethal chromosome aberrations such as translocations, and for not modelling complex aberrations explicitly.[38] However, the model has been acknowledged to be capable of predicting cell killing accurately even for doses as high as 25-50 Gy. This strength of the

LPL model made the model suitable for use in the study where the entire treatment course with doses as high as 66 Gy was modelled.

2.6 Conclusion

For all types of radiation therapy, a biological response is the ultimate goal. If the biological response is affected by temporal modulation, for example via repair mechanisms, this needs to be explicitly considered in treatment designs.

With regard to external beam radiotherapy, three significant time-related factors exist: the daily fraction duration, the duration of each fraction and the spatiotemporal dose-deposition pattern within each fraction. The latter two time scales describe intra-fraction dose effects and can be highly variable within each treatment fraction.

The biological effects of the temporal characteristics of IMRT are still poorly understood. In conformal radiation therapy, these temporal characteristics have neither been discussed nor explored in scientific literature. This might be due to the assumption that sub-lethal damage repair in a single fraction is negligible during the short treatment times used in conformal radiation therapy, compared to the long treatment times used in IMRT.

Studies regarding spatiotemporal effects on biological response have primarily been of a theoretical nature and have focused on overall treatment delivery time as a determinant of cell survival. *In vitro* studies have suggested a general pattern of sequencing the treatment fields in such a way that the low dose fields are treated first, followed by the high dose fields.

The studies also pointed out that variable time intervals between treatment fields in each treatment fraction can result in variable radiation damage. Other mathematically modelled studies[18, 19] support the results of *in vitro* studies. The effects observed during *in vitro* experiments by Lin *et al* and Benedict and modelled by Altman *et al* are attributable to the accelerated repair of radiation-damaged cells, which begins immediately upon irradiation and continues for two to four hours. During this time interval, the repair is initially very fast and non-linear, and then tapers off.[3] These dose-time effects have, so far, been observed *in vitro* and modelled in computational simulations for IMRT treatments. However, if these dose-time effects are applied to conformal radiotherapy techniques, then intra-fraction beam sequencing can be considered to affect treatment outcome.

Chapter 3

Research Methods

3.1 Introduction

In Chapter 2, the gap in literature regarding the possible radiobiological effect of temporal effects during each treatment fraction, when using conformal radiotherapy techniques, was highlighted. Consequently the study aimed to compare tumour cell radiosensitivity between the different beam sequences: those suggested by other studies, as well as those used in clinical practice. In order to reach the aim of the study, a quantitative research approach, as illustrated in Figure 3.1, was adopted.

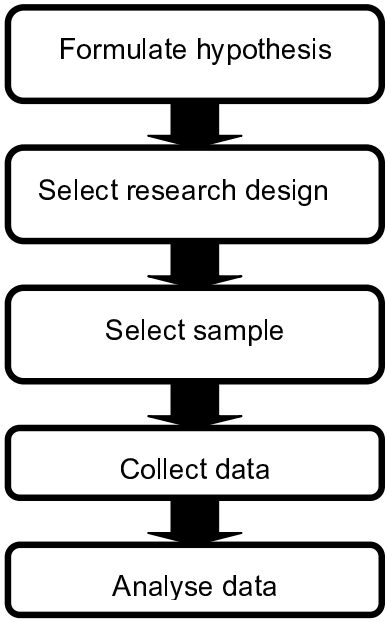

Figure 3.1: Flow chart of the research steps

3.2 Research design

A research design is a blueprint for conducting the study that maximises control over factors that could interfere with the study's desired outcome.[20] The research design directs the selection of the population, the sampling procedure, methods of measurement and the plan for data collection and analysis.[20] This study aimed to compare tumour cell radiosensitivities between three dose-delivery patterns: the triangular, the V-shaped and the conventional pattern for three prostate α/β ratios (i.e. 1 Gy, 1.5 Gy and 3 Gy). Therefore, a factorial experimental design was chosen, with the two factors being the dose-delivery pattern and the α/β ratio.[20] Each factor has three levels, thus making it a three-by-three factorial design, as shown in Table 3.1.

A factorial experiment allows for the simultaneous application of all possible level combinations of a given number of factors.[39] An advantage of this approach is that any significant interactions between the factors at the levels investigated are exposed, which is something that could be missed in an experiment varying one factor at a time.[40, 41, 42]

Table 3.1: Illustration of the study design

		α/β ratios		
		1 Gy	1.5 Gy	3 Gy
Dose delivery patterns	Triangular pattern	**X**	**X**	**X**
	V-shaped pattern	**X**	**X**	**X**
	Conventional pattern	**X**	**X**	**X**

3.3 Population

The current study is a modelled study performed using (1) clinical data (beam weighting values, degrees of beam directions) from clinical prostate treatment plans and (2) biological data (prostate tumour cell kinetic parameters) from the literature to perform computational simulations. The population of all prostate 3-D conformal treatment plans, created at the Radiation Oncology Department of Steve Biko Academic Hospital, which fulfilled the inclusion criteria was thus sequentially included in the study until the desired sample size of 45 was reached.

3.4 Eligibility criteria of the treatment plans

The clinical data used was collected from clinical treatment plans which were selected based on:

1) The tumour type, i.e. treatment plans created for prostate tumours;
2) Dose per fraction, i.e. 2 Gy per fraction used in the creation of the treatment plan;
3) The treatment technique, i.e. 3-D conformal treatment technique; and
4) Number of treatment fields, i.e. six treatment fields.

The (1) type of tumour and (2) dose per fraction have been identified by Altman *et al* [19] as the most important of the factors that interact with temporal optimisation of dose, where tumours whose α/β ratios approximate that of late-responding normal tissues (i.e. low α/β ratios) benefit more from this rearrangement than tumours with high α/β ratios;

and treatment plans where high doses per fraction, i.e. 3 Gy and above, were used were shown to be more sensitive to the dose-delivery rearrangement in IMRT.[19] Prostate tumour volumes planned and treated with IMRT were thus excluded from the study.

3.5 Sample size

By convention, in factorial experiments, sample size is chosen in such a way that the residual (error) degrees of freedom exceed 30.[20, 43] In this study, a sample size of 45 patient treatment folders was used, i.e. five patient treatment folders per α/β – dose-delivery pattern combination. Also, due to the fact that three or more variables are being considered in the study, an interaction term was included, giving 36 degrees of freedom for error.

3.6 Sampling technique

The sampling technique used was stratified sequential sampling because only the treatment plans which fulfilled the eligibility criteria, outlined in Section 3.4, were sequentially included in the study until the desired sample size of 45 was reached.

3.7 Research instrument

As mentioned in the previous sections, the current study is a modelled study conducted using computer simulations. To aid in the understanding of the specific research instrument, an overview of computer simulations is briefly given.

3.7.1 Overview of computer simulations

Computer simulations use models to imitate real life or to make predictions. To simulate something physical, first a mathematical model is created which represents that physical object. In order to create a model, a number of input parameters are required. The next task, once a model has been developed, is to execute the model on a computer, using algorithms to give outputs or response variables, as illustrated in Figure 3.2.

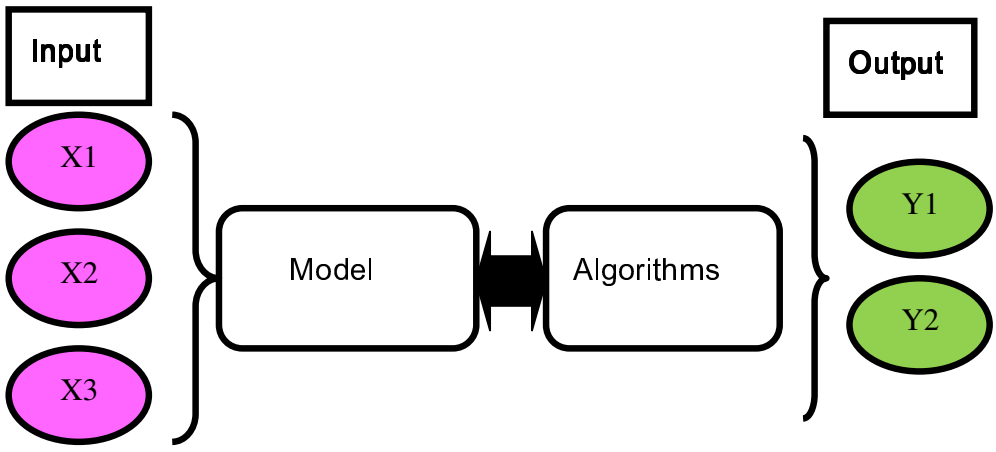

Figure 3.2: Diagrammatic illustration of a computer simulation

3.7.2 Simulation programme used in the study

This study was conducted using the Virtual Cell (VC) radiobiology programme Version 2, developed by Dr RD Stewart and his team at the School of Health Sciences, Purdue University. Dr RD Stewart is the Associate Professor and Assistant Head of Health Sciences, as well as the Director of Radiological Health Sciences at Purdue University. He has over 18 years of experience in Monte Carlo radiation transport, and has been a physics councillor and associate editor for the *Journal of Radiation Research* from 2007 to date.

The simulation programme is an ongoing effort to construct a multi-scale system of models to simulate early events and processes involved in pathogenesis and the treatment of cancer. Although the main biological endpoints of interest in the programme are cell death and neoplastic transformation, the VC programme also provides information regarding the formation and repair of DNA damage and information regarding chromosome aberration yields, the induction of genome instability, cell cycle kinetics and the probability of tumour eradication that follows radiation therapy.[44, 45]

Features of the programme include:

- Models (LPL, TLK, RMR) to track the formation and repair of 102 distinct types of DNA damage;

- Pathway-specific DNA repair models for single and multiple damaged DNA sites and DSBs;
- Monte Carlo Damage Simulation (MCDS) and Monte Carlo Excision Repair (MCER) algorithms to generate DNA damage configurations and repair data for both low and high LET radiation;
- Prediction of LET effects, oxygen effects and cell repopulation effects;
- Calculations of TCP, normalised iso-effective dose (NID), biologically equivalent dose (BED) and LQ parameters.[46, 47]

The two Monte Carlo algorithms used by the VC programme, i.e. MCDS and MCER, were validated by comparing their predictions to experimental data for low and high LET radiations. The comparisons suggested that both MCDS and MCER can be used to simulate end points (e.g. cell survival) related to the damage and repair of DNA in bacterial and mammalian cells.[45, 46]

3.8 Validity and reliability of the simulation model and parameters

In order to ensure validity of the results produced by the simulation programme, and to validate the simulation parameters used, the following measures were taken.

A.

Surviving fractions for split dose irradiation for low LET radiation were simulated. These simulations were performed over a range of doses, i.e. 2 Gy (1+1 Gy) to 10 Gy (5+5 Gy), separated by the duration of one hour to 15 hours as shown in Figures 3.3 to 3.5. Simulations were performed for cellular damage corresponding to dose-response curves with α/β ratios of 1 Gy, 1.5 Gy, 3 Gy and 10 Gy.

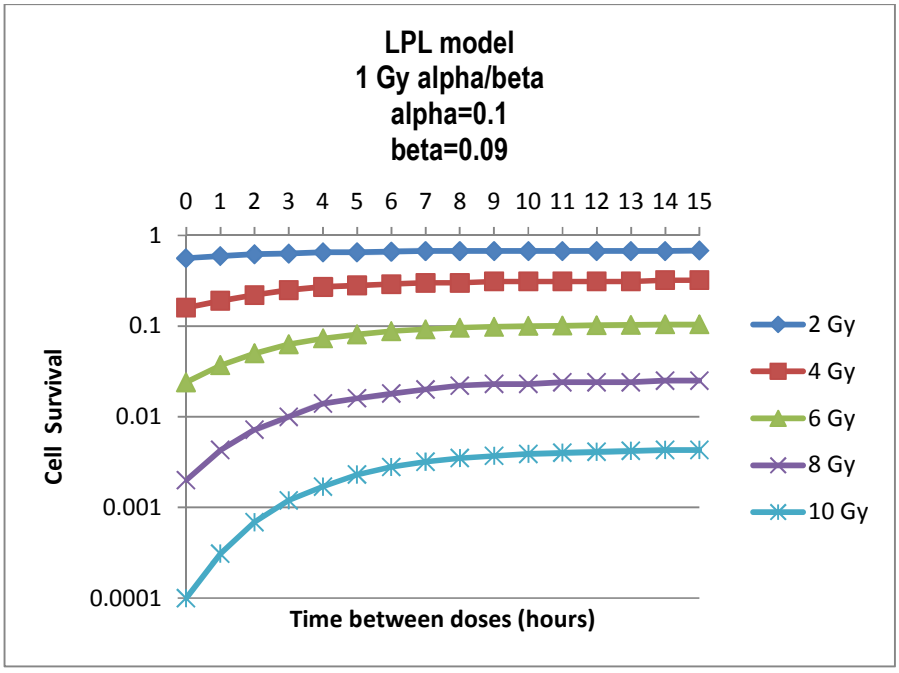

Figure 3.3: A plot of surviving fraction against time for split dose irradiation with an α/β ratio of 1 Gy

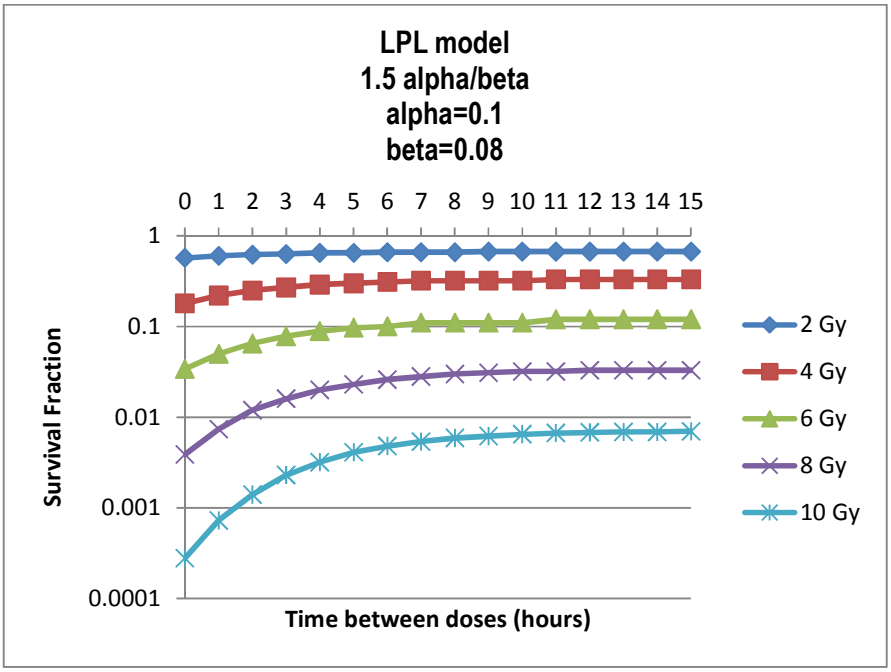

Figure 3.4: A plot of surviving fraction against time for split dose irradiation with an α/β ratio of 1.5 Gy

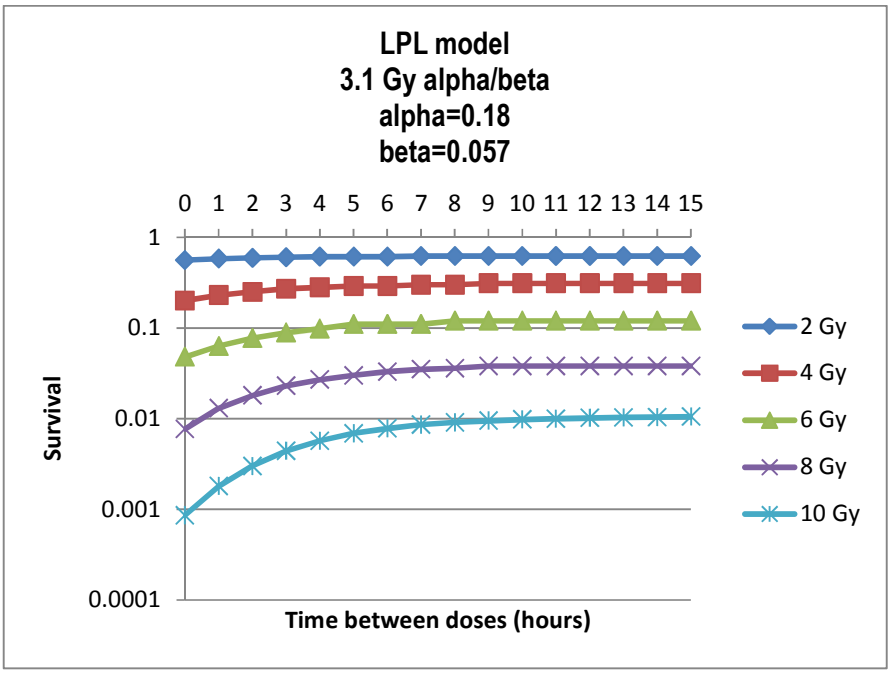

Figure 3.5: A plot of surviving fraction against time for split dose irradiation with an α/β ratio of 3 Gy

Since the study bases its argument on the repair of sub-lethal damage, recovery ratios of the mentioned α/β values and dose ranges were also calculated using the equation:

$$\text{Recovery Ratio (RR)} = \frac{S(t)}{S(0)} \qquad (3.1)$$

Where S(t) is cell survival at time (t) between doses, and S(0) is cell survival at time zero between doses. The recovery ratios were found to increase as the dose increased as is evident in Figure 3.6. This is the most important validation given the context of the study. Furthermore, if the simulations are accurate, the relationship between the recovery ratios and the dose per fraction should result in a straight line, as is evident in Figure 3.6.

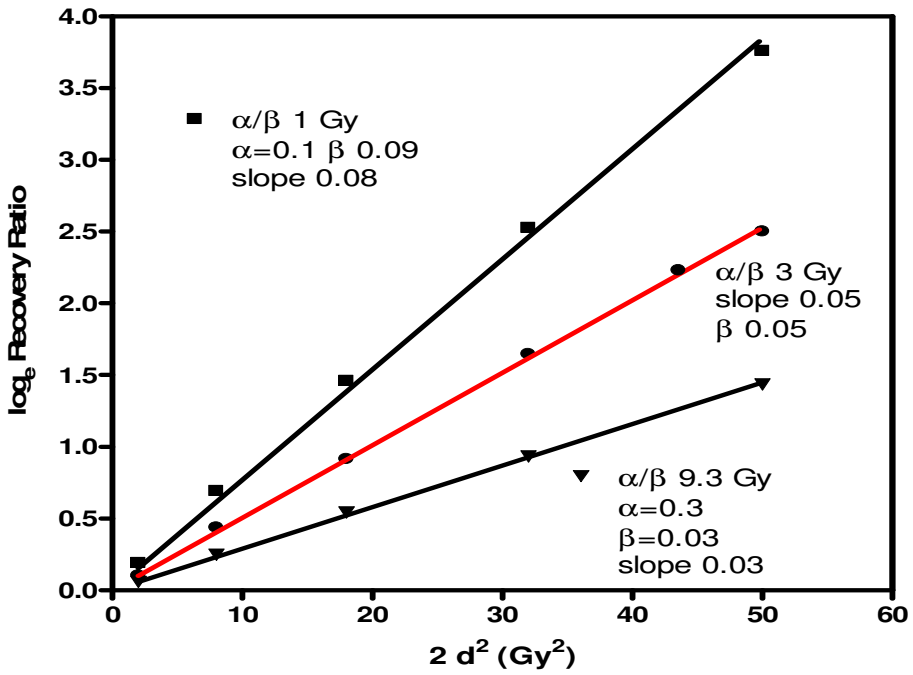

Figure 3.6: A plot of the log of recovery ratio against two times the dose-squared for three α/β ratios used in the test simulations

The slope of the simulated split dose cell survival curves was calculated by squaring the dose and multiplying it by two, i.e. ($2d^2$) and plotted against the log of recovery ratio.[21] To reflect the accuracy of the simulated cell survival curves, the slope should approximate the predicted beta value. Figure 3.6 indicates that, in each case, the slope of each curve closely approximated the beta value for the acute survival curve constructed from the simulations. Also, the recovery ratio increased with the dose as expected.

B.

Split dose irradiation simulations and recovery ratio calculations using high LET radiation (80 keV per micron) were performed and estimates of the Mean Inactivation Dose (MID) for the resultant cell survival curves from both low LET and high LET simulated data were calculated (Table 3.2).

Table 3.2: Estimates of the MIDs for the three α/β ratios

	Low LET		High LET
	α/β = 1 Gy	α/β = 10 Gy	80 keV/micron (Neutrons)
Alpha (/Gy)	0.158	0.315	0.869
Beta (/Gy)	0.0766	0.0285	0.0347
Mean Inactivation Dose (Gy)	2.39	2.35	1.1

Furthermore, to validate the accuracy of the high LET simulations, Neutron relative biologic effectiveness (RBE) for the two extreme α/β ratios (i.e. 1 Gy and 9.3 Gy) was expressed in relation to photons:

For a 1 Gy α/β ratio:

$$RBE = \frac{\text{Mean inactivation dose of x-rays}}{\text{Mean inactivation dose of Neutrons}}$$

$$= \frac{2.39}{1.1}$$

$$= 2.2\ Gy$$

For a 9.3 Gy α/β ratio:

$$RBE = \frac{\text{Mean inactivation dose of x-rays}}{\text{Mean inactivation dose of Neutrons}}$$

$$= \frac{2.35}{1.1}$$

$$= 2.1\ Gy$$

These RBE values calculated from the simulated data were consistent with that obtained from *in vitro* experiments.[48, 49]

3.9 Data-collection process

Data collection was performed in three phases, i.e. Phase A, Phase B and Phase C.

Phase A: Preparation of input data

Step 1: Reviewing the treatment field sequence

Patient treatment plans created for prostate tumours, using six treatment fields which fulfil the inclusion criteria, were reviewed by the researcher. For each treatment plan, the treatment beam sequence used to treat the fields at the treatment unit was identified from the treatment folders and recorded. While reviewing the entire sample, it emerged that the most common pattern followed in the treatment delivery of prostate was to treat in a clockwise direction from the right posterior oblique, right lateral, right anterior oblique, left anterior oblique, left lateral to left posterior oblique, as shown in Figure 3.7. This pattern was adopted as the conventional pattern for use in the study.

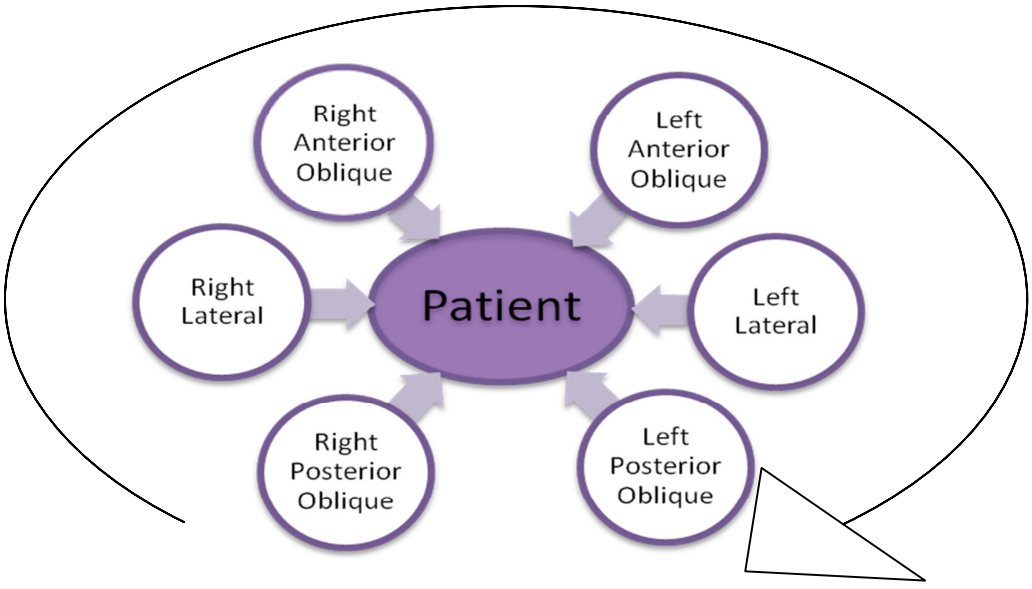

Figure 3.7: Illustration of the conventional pattern used in the study

Treating in a counter-clockwise direction, i.e. from the left posterior oblique and ending with the right posterior oblique, was followed in ten per cent of the reviewed patterns.

Treatments given in a counter-clockwise direction result in the same dose-delivery pattern as when applied in a clockwise direction because the beam weightings of the two posterior oblique fields, the two lateral fields and the two anterior oblique fields are always equal.

Step 2: Reviewing the beam weightings

The beam weightings that correspond to each treatment field for each treatment plan were also reviewed. After reviewing the treatment beam weightings for each treatment field and for each treatment plan, only eight different beam weight combinations in cGy, shown in Table 3.3, were identified. That is, the beam weight combinations repeated themselves.

Table 3.3: Different treatment beam weighting combinations in cGy for corresponding beam positions

	Treatment field positions						
Beam weighting combinations	No	RPO	RLAT	RAO	LAO	LLAT	LPO
	1(n=20)	31	42	32	31	42	31
	2(n=11)	35	45	35	30	45	30
	3(n=4)	36	37	36	32	37	32
	4(n=1)	37	31	37	37	37	31
	5(n=2)	40	42	40	35	42	35
	6(n=3)	30	40	30	40	30	30
	7(n=2)	31	35	34	35	31	34
	8(n=2)	31	34	33	34	33	33

* RPO/LPO – Right/Left Posterior Oblique, RLAT/LLAT – Right/Left Lateral, RAO/LAO – Right/Left Anterior Oblique

Step 3: Rearranging the beam sequences to form the experimental triangular and V-shaped patterns of dose delivery

Lastly, from the eight commonly used beam weighting combinations the beam weightings of each treatment field were sequentially rearranged to form the triangular and V-shaped patterns for dose delivery for purposes of the study. The rearrangement was conducted as suggested by Altman *et al.*[19] In the triangular pattern, the two highest beams were selected and set to be applied in the middle of the fraction, with the highest field last. The next two highest fields were then selected, with the larger of the two set to be applied after the previous highest dose field. This process was repeated until all fields had been placed.

In rearranging the beam weightings to form the V-shaped pattern for an even number of beams the two lowest dose beams were selected and placed as the middle two fields, with the lowest of the two set to be applied first. The remaining beams were ordered by calculating two "half-pattern weights," which represent the sums of the doses currently placed in the first half and second half of the fraction. The lowest non-placed beam was

set to be applied before or after the half of the plan with the highest current weight. This process was also repeated until all beams had been placed.[19]

Step 4: Creating the input file

In preparation for the simulation process, the researcher created input files to be read by the VC simulation programme, Appendix A. The input files contained data fields for the biophysical parameters, cell repair parameters, damage formation parameters, and cell biological parameters of prostate tumour cells, as well as the exposure set-up and simulation conditions. A detailed description of the simulation parameters and sources used in the input files is provided in Appendix B.

Phase B: Performance of simulations

The Virtual Cell is a batch-style computer programme that has been written in Fortran 90/95 computer language and does not have a Graphical User Interface (GUI). The simulations were, therefore, performed from the Microsoft Windows command prompt. First, the expected damage configurations were generated using the Monte Carlo Damage Simulation (MCDS) algorithm. The generated damage configurations were then specified in the input file (DSB parameter). The Monte Carlo Excision Repair (MCER) algorithm was invoked to simulate repair, misrepair and aborted excision repair of damage within the entire genome or within a specific region of DNA, as illustrated in Figure 3.8.[47, 50]

Lastly, the repair data generated by the MCER algorithm was used by the programme to compute damage repair kinetics, mutations, genomic instability, cell killing and neoplastic transformation.

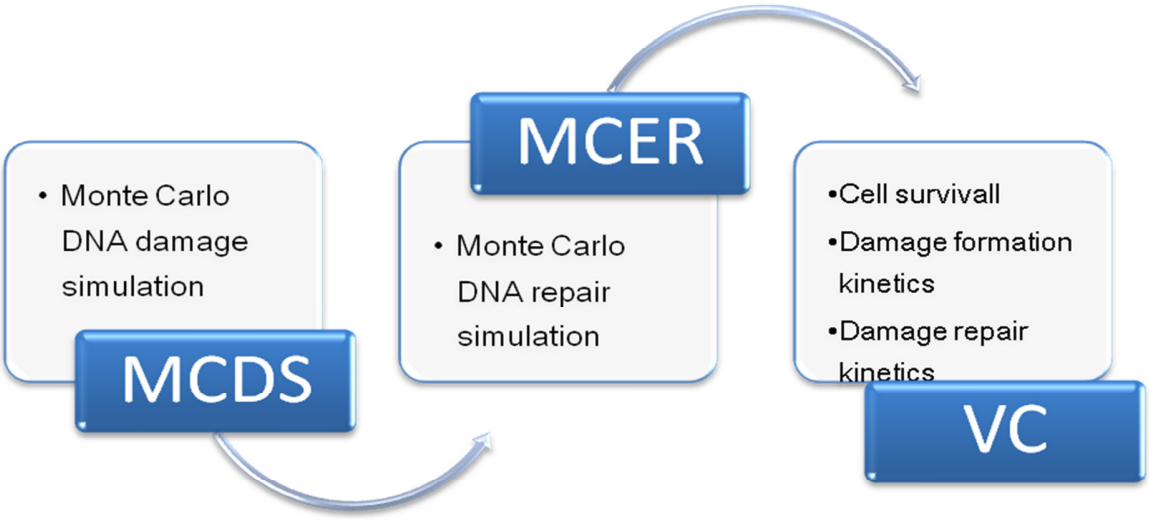

Figure 3.8: Illustration of the simulation process[47]

Phase C: Output results

The output file created by the simulation programme contains information given in Appendix C. This includes information on single-strand break damage formation and repair, double-strand break damage formation and repair, cell and tissue kinetic parameters, cell survival as a function of dose and dose rate, cell survival corrected for repopulation effects, mutagenesis and induction of enhanced genetic instability,

neoplastic transformation as a function of dose and dose rate, and tumour control estimates as a function of dose and dose rate.

3.10 Ethical considerations

The study did not involve any human participants, which meant that the ethical principles of beneficence, non-maleficence, fidelity, justice and veracity were not applicable. In order to satisfy the relevant principle of confidentiality, patient identity on all treatment plans was masked. Authorisation was also obtained from the Steve Biko Academic Hospital Chief Executive Officer and Head of the Radiotherapy Department to access the clinical radiotherapy treatment folders. All data will be stored for a period of 15 years to comply with good clinical research practice guidelines.

3.11 Data analysis

MID was used to analyse cell survival data and to pick up differences in radiosensitivities between the three α/β ratios when using the different dose-delivery patterns. The concept of MID was introduced as early as 1972 by Keller and Hugo[52], and is to date recommended by the International Commission of Radiation Units and Measurements (ICRU) for use in analysing survival curves of mammalian cells.[53] MID is defined as the average dose of the differential survival probability distribution, and is expressed by the equation:

$$\bar{D} = \int_0^\infty S(D)\, dB \qquad (3.2)$$

Where S(D) is the survival probability at dose D. The Mean Inactivation Dose (D) thus equals the area under the survival curve plotted on linear scale/coordinates.[54] Cell survival fractions (S) as a function of dose (D) were fitted to the LQ equation as $\log_e(S) = -\alpha D - \beta D^2$. The parameters α and β were obtained by regression analysis of the logarithm of S. In cases where one of the inactivation parameters (α or β) was found to be negative, it was set to zero and the other parameter was re-estimated. The goodness of fit was then verified and the LQ function was then integrated using the 12-point Gauss formula to obtain MID.[49]

Compared to other methods of representing radiosensitivity (e.g. Do and extrapolation number (n)), MID is preferred because:

- Significant differences in radiosensitivity between categories emerge when using MID.
- MID is representative of the whole cell population rather than of the fraction of it.[56, 57]

MIDs for the entire survival curve for each α/β ratio and for each dose delivery pattern were calculated.

Furthermore, the Kruskal Wallis non-parametric statistical procedure was used to test whether differences in MID between the dose-delivery patterns and between the different α/β ratios was statistically significant. Specific differences were tested for, with appropriate contrasts. Hypothesis testing was carried out at the 0.05 level of significance.

3.12 Conclusion

This chapter outlined the research approach used to fulfil the aim of the study. The study design used to either accept or reject the null hypotheses was outlined. The methods of data collection used and the data-collection instrument were also described in detail. The ethical considerations relevant to the study were also addressed. Lastly, the statistical methods used to analyse the data were discussed. The results of the analysis are presented in Chapter 4.

Chapter 4

Results

4.1 Introduction

In this chapter, the results are presented in tabular and narrative form. Additional data that could be used for interpretation of the data is included in Appendix C.

4.2 Description of the sample

The sample consisted of 45 treatment plans created for prostate tumours, using 3-D conformal technique, six treatment fields, a dose per fraction of 2 Gy and a dose rate of 200 cGy/minute. Owing to the fact that the specific α/β ratios of the individual patients were not determined, three α/β ratios of prostate tumours were simulated and used in order to cover the applicable range of suggested prostate α/β ratios.[33] As mentioned in Section 3.9, of the 45 treatment plans, eight different beam weight combinations were identified. For purposes of the study, analysis of cell survival among the eight different beam weight combinations was conducted, and the results yielded a non-significant difference with p=0.999. Therefore, only one beam weight combination, i.e. the most employed, was used to compare cell survival between the three dose-delivery patterns, among the three α/β ratios.

4.3 Comparison of the Mean Inactivation Dose between dose-delivery patterns

MIDs for the three dose-delivery patterns resulted in the same value (Table 4.1).

Table 4.1: MIDs for the three dose-delivery patterns

		α/β ratios		
		1 Gy	1.5 Gy	3 Gy
Dose delivery patterns	Triangular pattern	2.48 Gy	2.30 Gy	2.23 Gy
	V-shaped pattern	2.48 Gy	2.30 Gy	2.23 Gy
	Conventional pattern	2.48 Gy	2.30 Gy	2.23 Gy

An analysis of MIDs revealed a non-significant difference with p=0.5436 between the three dose-delivery patterns.

4.4 Comparison of the Mean Inactivation Dose between the three α/β ratios

The MID was observed to change with α/β ratio (Figures 4.1 to 4.3).

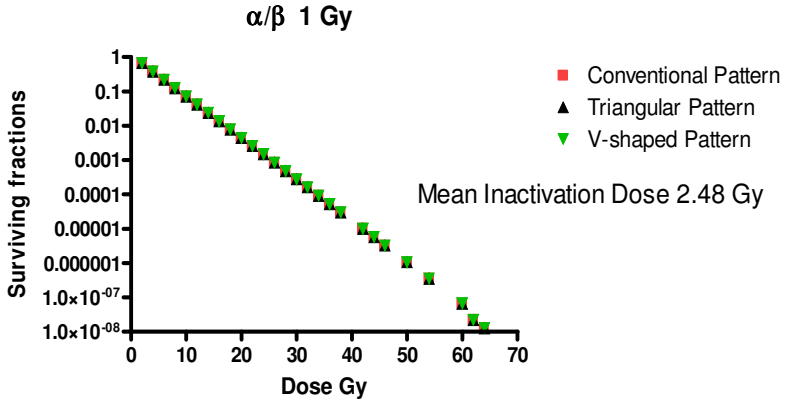

Figure 4.1: Cell survival curve for the three dose-delivery patterns at 1 Gy α/β ratio

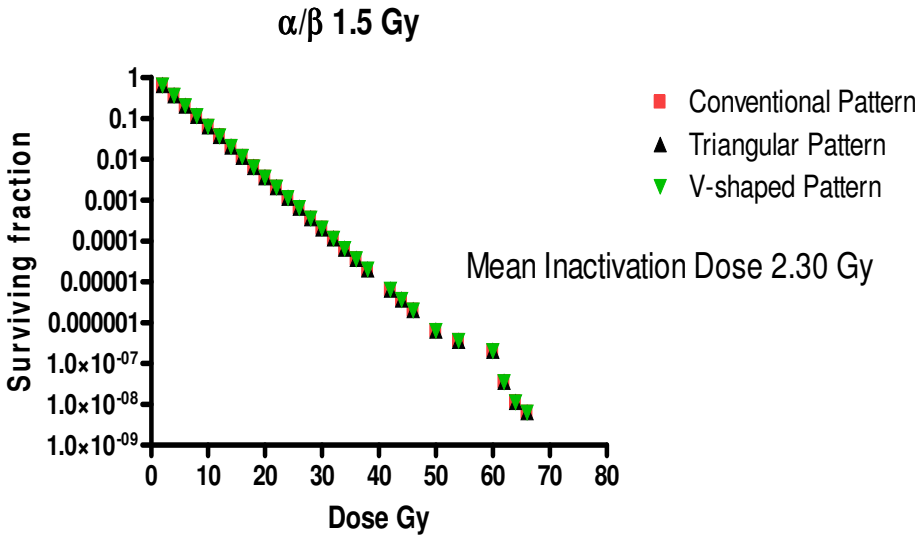

Figure 4.2: Cell survival curve for the three dose-delivery patterns at 1.5 Gy α/β ratio

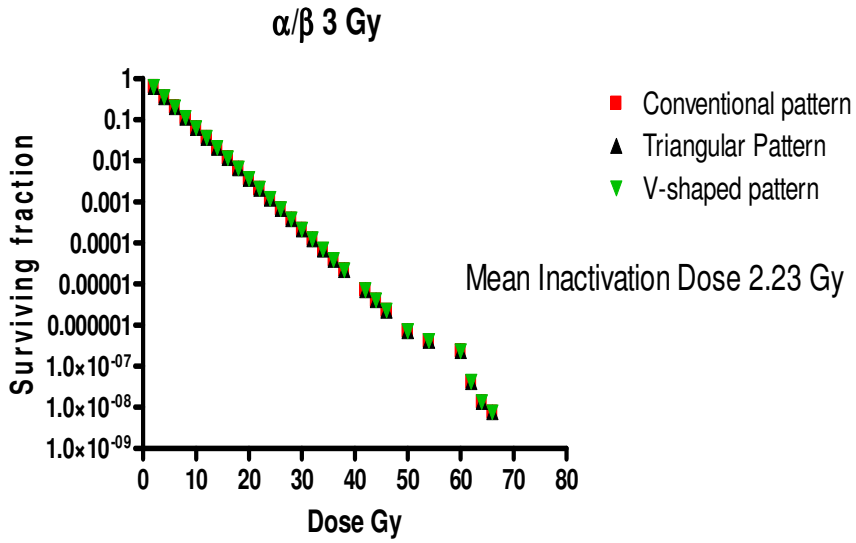

Figure 4.3: Cell survival curve for the three dose-delivery patterns at 3 Gy α/β ratio

Using MID, a statistically significant difference with p= 0.0183 was found between the three α/β ratios.

4.5 Results in relation to hypotheses

Null hypothesis$_1$ postulated that there is no significant difference in MIDs between the triangular and conventional patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques. The results show a non-significant difference, with p=0.5436. Null hypothesis$_1$ is thus accepted.

Null hypothesis$_2$ postulated that there is no significant difference in MID between the triangular and V-shaped patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios using conformal radiotherapy treatment techniques. The results indicate that the triangular pattern is not statistically different from the V-shaped pattern, with p=0.5436. Null hypothesis$_2$ is thus accepted.

Null hypothesis$_3$ postulated that there is no significant difference in MID between the V-shaped and conventional patterns of dose delivery when applied to prostate tumour cells with 1 Gy, 1.5 Gy and 3 Gy α/β ratios, using conformal radiotherapy treatment techniques. The results show a non-significant difference with p=0.5436. Null hypothesis$_3$ is, therefore, not rejected.

4.6 Conclusion

This chapter presented the main results of the study. From the results it is evident that the three patterns of dose delivery do not differ significantly across all three α/β ratios used. A significant difference in MID noted between the α/β ratios indicates that as the α/β increases, i.e. as radiosensitivity increases, MID, which quantifies the radiosensitivity of cells, decreases. Possible reasons behind these findings are discussed in Chapter 5.

Chapter 5

Discussion

5.1 Introduction

Chapter 1 presents the research problem and aims of the research, and Chapter 2 provides a review of relevant literature. Chapters 3 and 4 outline the research methods used to reach the purpose of the study, and present the results of the investigation. In this chapter, the results presented in Chapter 4 are interpreted and possible reasons for the findings are discussed. This chapter aims to draw together the research findings and summarise the main interpretations arising from them.

5.2 Summary of the main findings

The results of the study indicate that the biologic effect (expressed in terms of MID in the study) of different beam sequences does not vary significantly with the sequence in which the beams are ordered and treated. The results further indicate that the biologic effect does vary with the tissue type, with most biologic damage (i.e. low MID) observed at higher α/β than at low α/β values.

5.3 Analysis of Mean Inactivation across the three dose-delivery patterns

The observed non-significant differences in MIDs between the dose-delivery patterns is thought to be mainly attributable to the biological and technical facts discussed in the following sections.

5.3.1. Treatment times that are shorter than sub-lethal damage repair half-times

As mentioned in Section 1.2, in conformal radiation therapy, fraction delivery times normally range between two and five minutes, depending on the number of fields being treated. According to Wang *et al*[6], when fraction delivery times are comparable to or longer than the half-time repair of the cells being irradiated, sufficient time is allowed for sub-lethal damage repair to take place and cell killing is decreased. For human tumour cells, half-times for sub-lethal damage repair, T ½, which is the time needed for half the repair to take place, has been estimated to range from a few minutes to several hours.[6] More specifically, Steel *et al* estimated half-time repairs of 0.36 hours (21.6 minutes) to 2.3 hours for mammalian cells.[55] For prostate tumour cells, a repair half-time of 16 minutes was determined by Wang *et al*[6] from clinical data. Similarly, Steel *et al*[55] determined a repair half-time that ranged from 15 minutes to one hour from split dose experiments.[55]

However, it has been observed that repair half-times determined from split dose experiments tend to be longer than repair half-times estimated from continuous low

dose rate experiments, where repair half-times in the range of six minutes to 54 minutes have been observed.[56] In the current study, an analysis of repair half life for surviving fractions for split dose 5 + 5 Gy for tissue with an α/β ratio of 1 Gy was also carried out. The fitted line corresponds to a half-time repair of 12 minutes (Figure 5.1).

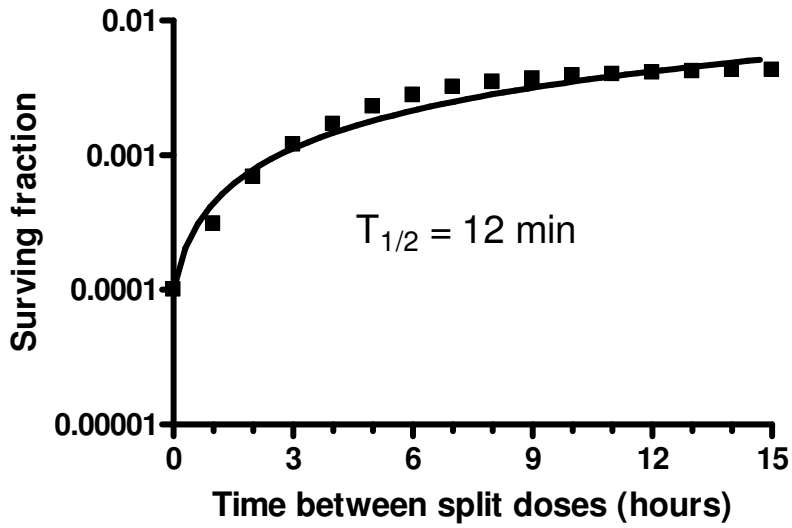

Figure 5.1: Surviving fractions of cells exposed to split dose radiation of 5 Gy plus 5 Gy separated with different times

The estimated repair half-times, either from literature or the one determined in the current study, are clearly comparable to treatment delivery times typically used in IMRT but not to the treatment times used in conformal radiotherapy techniques. In the current study, a treatment delivery time of three minutes, as observed in clinical practice, was used to simulate all the dose-delivery patterns. This value is below the determined

repair half-time of 12 minutes, hence the observed non-significant difference in cell survival between the three dose-delivery patterns.

If the above argument stands true, this raises the question: when does repair of radiation-induced cellular damage begin after irradiation? According to Lin *et al*[2], the fast repair component of DNA damage is in the order of minutes. More specifically, the formation of a repair biomarker has been observed in irradiated cells within three minutes. In the current study, interval time of one minute between treatment fields was used in the simulation. This interval time was chosen consistent with the time observed in clinical practice. Clearly, a one-minute interval time is less than the observed three minutes required for the fast repair component to commence.

5.3.2. Constant high dose rates used in conformal radiotherapy treatment techniques

Another factor that is thought to have contributed to the non-significant cell survival differences between the dose-delivery patterns is the constant high dose rate used in conformal radiotherapy treatment techniques. From clinical practice, dose rates of 200 cGy/minute, i.e. 2 Gy/minute or 120 Gy/hour, were observed to be used. According to the International Atomic Energy Agency (IAEA)[57], repair during irradiation is negligible at the high dose rates of 1-5 Gy/min, i.e. 60-300 Gy/hour, practised in external beam

therapy and high-dose-rate brachytherapy, but is very significant during the course of the 1.6-150 cGy/min practised in lower-dose-rate brachytherapy[57].

In the background to the study, it was stated that the study was undertaken in response to the assertions of the Altman *et al* study that the V-shaped and triangular patterns of dose delivery can be used to vary tumour cell kill in IMRT and conformal radiotherapy techniques as well. In their study, the authors used IMRT cases, which use variable dose rates ranging from low to high dose rates. This suggests that it can be assumed that the low dose rates used in some treatment fields of an IMRT treatment are the rates responsible for the differences in repair which resulted in the difference between the dose-delivery patterns, as observed in the Altman *et al* study.

5.3.3. Size of individual treatment field doses

Lin *et al*[2] argue that if the number of treatment fields in each 2 Gy fraction is greater than five, i.e. if the dose of individual fields is less than 0.5 Gy, the influence of total fraction delivery time on cell survival becomes small because of low dose hyper-radiosensitivity (HRS). That is, at doses of less than 0.5 Gy per field, the cells are hypersensitive to radiation. This means that they suffer non-repairable damage and the role of cellular repair mechanisms which are determined by the total fraction delivery time is very limited. However, at doses that are greater than 0.5 Gy per field, increased

radio resistance (IRR) dominates, rendering the cell more radio resistant because of the activated repair mechanisms.[2]

In the current study, six treatment fields were used in each treatment fraction, with individual dose fields ranging between 0.3 Gy and 0.4 Gy in each fraction. Therefore, one would assume that, because HRS dominates at doses of less than 0.5 Gy per field, HRS dominated in each treatment fraction in the first two to three fractions, and, during this time, the influence of fraction delivery time on cell survival was insignificant.[2] Furthermore, one would be justified in further assuming that in subsequent treatment fractions, i.e. treatment fraction 4 to 33, IRR dominated because the accumulated doses from previous treatment fractions would be larger than 0.5 Gy per treatment field and the induction of IRR would have induced the repair mechanisms. However, with the short treatment times used in the study, insufficient time is allowed for these repair mechanisms to have any significant influence on cell survival; hence, similar MID values across the three dose-delivery patterns were observed.

5.4. Analysis of Mean Inactivation Dose across the three α/β ratios

The observed differences in MIDs between the three α/β ratios indicate that as the α/β ratio increases the MID decreases. This observation is consistent with what is already documented; that tissues with high α/β ratios possess a decreased repair capacity, i.e.

low beta value; hence, they appear to be more radiosensitive. Tissues with a low α/β ratio, on the other hand, possess a high beta value, i.e. they have an increased repair capacity; hence, they appear to be radio resistant. The comparison made here between MID value and α/β ratio is done with great caution. The reader is reminded that MID, which is a simple measure of the radiosensitivity of cells (units in Gy), is a concept that is very different from an α/β ratio. The latter is the dose in Gy where the lethal and repairable components of radiation damage make equal contributions to the total level of tissue damage. The α/β ratio is in fact a variable that reflects radiosensitivity, i.e. the α-value divided by a β parameter which reflects the induction of repairable damage.

Although the differences in MID across the three α/β ratios are statistically significant, these differences are less important in clinical practice, where oxygen status and heterogeneity play a role. In the theoretical modelling used in the study, these factors do not influence results. Besides, the different α/β values for prostate used in the study, which represent intra-patient heterogeneity, have been found by Mooisenko[58] to have little effect on TCP. A closer scrutiny of these differences indicates that as the α/β value increases the difference in MID decreases, i.e. the difference in MID value of 1 Gy and 1.5 Gy α/β is more than the difference in MID values of 1.5 Gy and 3 Gy α/β ratios. This observation is thought to be due to a decrease in repair capacity as the α/β values increase.

5.5. Conclusion

The chapter outlined the possible reasons for the observed insignificant difference in MID across the three dose-delivery patterns, i.e. short fraction duration time used in conformal radiotherapy techniques, constant high dose rate used and the small size of individual dose fields used. The statistically significant differences in MID across the three α/β values used are not expected to have any clinical relevance.

Chapter 6

Conclusions and Recommendations

6.1 Introduction

In this chapter, the conclusions, as well as the relevance of the findings of the study, in terms of the contribution to scientific knowledge and applied practice, are presented. The interpretations of the results, as discussed in the previous chapter, are clarified and the merit of the research is critically appraised. In essence, the conclusions to the research question and hypotheses postulated in Chapter 1 are presented. Implications of the research results for clinical practice and recommendations for further research are also presented.

6.2. Main conclusions

The study determined that the triangular and V-shaped patterns of dose delivery do not result in a different biological effect when applied to 3-D conformal treatment techniques. The current study further compared these recommended dose-delivery patterns to the conventional patterns used at the Radiation Oncology Department of Steve Biko Academic Hospital. The conventional pattern resulted in a MID value that is comparable to that of the V-shaped and the triangular pattern. This was because the

fraction treatment times, constant high dose rates and individual dose fields used do not favour the differential sub-lethal damage repair.

Although only one beam weighting combination was used to perform this theoretical modelling, the results can be generalised to all the different beam weighting combinations used in clinical practice because, as shown in Table 3.3, a very small difference exists between these individual dose fields. Everything considered, this small difference is not expected to result in a noticeable difference in biologic effect. A statistical analysis of cell survival between these different beam weight combinations further confirmed this non-significant difference.

Also, it should be acknowledged that in clinical practice different conventional patterns may exist. Figure 6.1 shows the different conventional patterns used in the research setting. These different conventional patterns were also statistically confirmed to have non-significant differences in biologic effect.

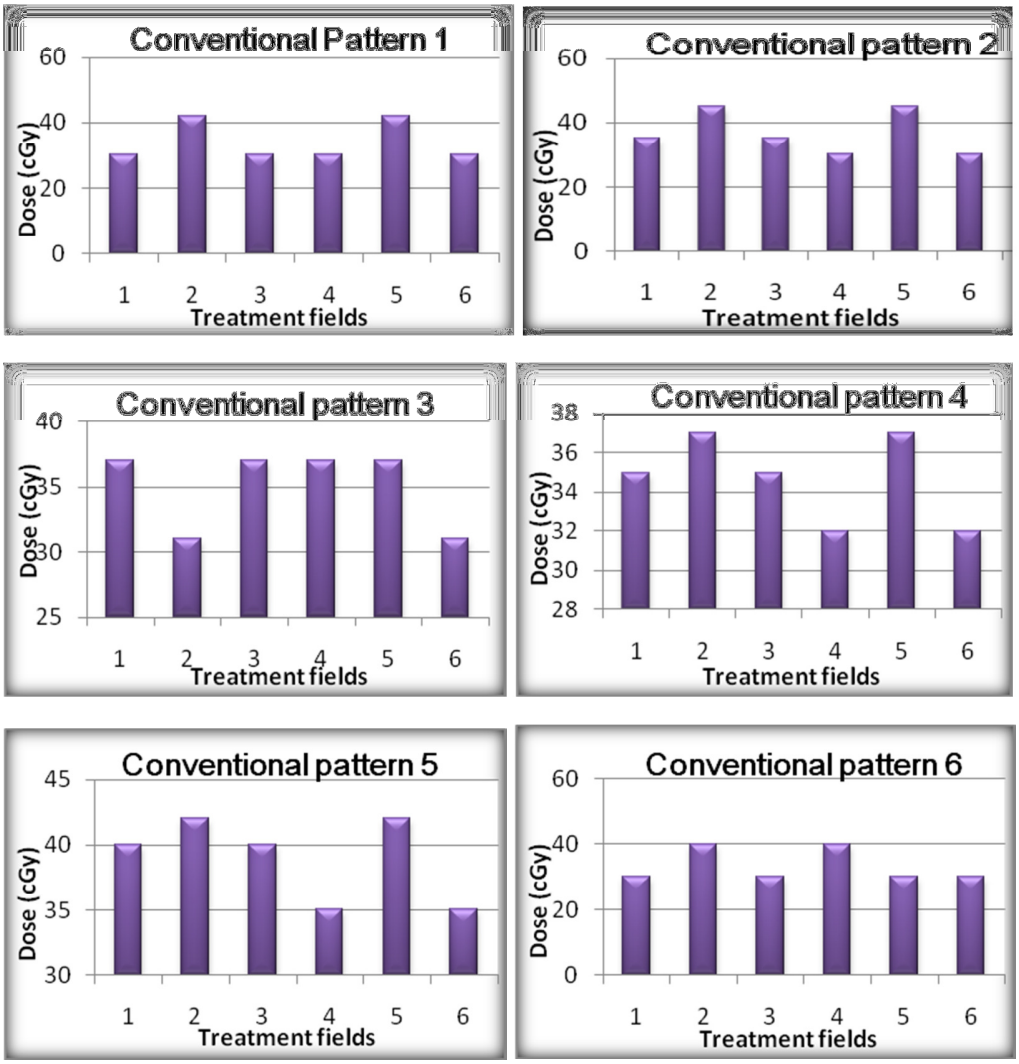

Figure 6.1: Diagrammatic presentation of the conventional patterns used in clinical practice

Furthermore, the results of the study can be generalised to tumour types with high α/β ratios as well. This generalisation is based on the fact that tumours with high α/β ratios possess a low repair capacity for repairing radiation damage. If the difference in biologic effect between the dose-delivery patterns is mainly due to sub-lethal damage repair,

clearly this difference is not expected to be evident in the high α/β ratio tumours because of the low sub-lethal damage repair capacity.

6.3 Implications for clinical practice

For conventional conformal radiotherapy treatment techniques, the current practice of sequencing treatment beams does not need to be changed. For IMRT, the sequencing of beams in a triangular and V-shaped pattern to enhance tumour cell kill in tissue types with low α/β ratios should be considered.

6.4. Evaluation of the research

In experimental studies internal validity helps to determine cause and effect relationships, and to conclude whether the changes observed in the dependent variable are caused by changes in the independent variable. External validity help determine whether the study results can be generalised to other groups or settings. For the current study, internal and external validity is evaluated in section 6.4.2 and 6.4.2

6.4.1 Threats to internal validity

In experimental designs, internal validity of the research process is essential to allow the researcher to draw accurate conclusions regarding cause and effect from the yielded data. To maximise internal validity, the researcher controlled extraneous variables so that these variables could be ruled out as an explanation for any effect

observed. A standardised set of procedures was followed during all simulations for each of the dose-delivery patterns. All simulation parameters (i.e. prostate cell kinetics parameters, radiation exposure set-up scenario and simulation conditions) were kept the same across the dose-delivery patterns. The fact that the elements were used as their own control helped to eliminate assignment bias.

The sample size was large enough to address variations caused by experimental errors. In the current study, experimental errors may partially occur due to the probabilistic nature of radiation interaction. That is, even irradiation with the same dose, using the same geometric parameters, does not mean that the number of cells with which the radiation interacts will be the same each time. Even if the given numbers of cells are "hit" with radiation, the radiation does not necessarily interact with the DNA to cause critical damage. Furthermore, cell survival over the entire treatment course was generated to take into account type-3 damage, i.e. sub-lethal damage that interacts with sub-lethal damage formed previously in the same fraction to form lethal damage. Therefore, the results presented are a reflection of what happens in clinical practice.

The researcher faced some challenges in finding the radiobiology simulation programme as most simulation programmes are focused on simulating the transportation of radiation particles. Among others, the programmes that were initially

recommended by medical physicists for use in the study included the Monte Carlo N-Particle (MCNP) transport code and the Geant 4 DNA. The Geant 4 DNA was better suited for the study than the MCNP, but was still in its early development stages. As a result of further enquiries, the Virtual Cell Radiobiology programme was acquired from Dr RD Stewart at the Purdue University in America and was used in the study. Although the programme used in the study has been validated and used in other studies, it was the researcher's first encounter with the programme. To counter this problem, as described in Chapter 3, test simulations to select the most valid simulation model for the study and to validate the results of the simulations were conducted under the supervision of an experienced radiobiologist.

With regard to the research instrument, the MCDS damage generation algorithm used in the simulation provides a simple and fast algorithm to simulate formation of single- and double-damaged DNA sites, including various classes of single strand breaks (SSBs) and DSBs. Although the algorithm successfully reproduces many of the small-scale damage-clustering effects predicted by track structure codes, the algorithm provides no information regarding where the lesions are located within the DNA of a cell. The algorithm also has a lower dose limit. To give meaningful results, the algorithm requires that DNA segment length ($n_{seg}D$) be much larger than the minimum length of undamaged DNA between neighbouring elementary damages (N_{min}). Dose (D) should

also be much larger than the ratio $N_{min}/n_{seg.}$ The algorithm is, however, expected to be accurate for doses as low as ~1 cGy. MCDS also does not provide any information on the yield of radiation-induced apurinic / apyrimidinic (AP) sites and this type of damage is not considered in the results of the study.[48, 51]

Within the MCER model, errors in DNA synthesis are assumed to yield single-base substitutions. Because of this model simplification, base substitutions cannot be distinguished from mutations or small deletions. Another limitation of the instrument is that the LPL model used in the study does not explicitly account for cell-cycle and other proliferation-related phenomena that are relevant to the analysis of some radiobiological experiments.[36, 37]

The analysis performed in this study provides an understanding of the biological effect of different dose-delivery patterns for the entire treatment course. There are, however, several caveats that require discussion:

- First, the treatment set-up is highly simplified. It is assumed that the duration of beams –on segments is equal and that the duration of moving the irradiator from one beam to the other is also equal.
- Second, the biological model, although commonly used for many *in vitro* and *in vivo* biologic processes, is quite simplified. Although some physical factors and

biological factors that determine the response of cells to radiation have been incorporated in the simulation model, e.g. LET, dose and repopulation effects and repair, some biological factors have been ignored, e.g. re-oxygenation, the position of the cell in the cell cycle, the rate of proliferation and the extent of differentiation.[19]

Although clinical data, as well as biological data, was used in the study, the results are based on mathematical predictions and may need to be validated clinically. Further studies, using *in vitro* and *in vivo* methods, are required to test the veracity of these results. Radiobiological models used in the study are useful in estimating the biological effects of radiation. However, their results should not be used to replace clinical judgement, but rather to supplement it.[58]

The LPL model used accounts for only one type of DNA damage. This is a limitation in that, given the random nature of radiation interaction with cells; even if the DNA in a cell is damaged the damage might be sub-lethal and not contribute to the observed endpoint, which is cell kill.

6.4.2 Threats to external validity

As mentioned in Chapter 5, the results of the conventional pattern used in the study can be generalised to other conventional patterns used in other clinical settings as different conventional patterns exist. During data collection, the inclusion of the conventional

pattern in the study was seen as a weakness in that several conventional patterns exist in a single clinical setting, as shown in Figure 6.1, as well as in different clinical settings, i.e. the conventional pattern cannot be standardised. However, the results of the triangular and V-shaped and conventional patterns used in the study indicate a similar biologic effect from all three dose-delivery patterns.

It should be noted, however, that even though only prostate tumour cells were simulated and not normal tissue cells, the results can also be generalised to other tumour types with low α/β ratios and normal tissues with low α/β ratios because their radiosensitivity parameters are similar.[18, 19] Also, even though three prostate α/β ratios were used in the study to cover the suggested range of prostate α/β given in the literature, caution should be applied when the results of the study are generalised to all prostate tumours because evidence confirming low α/β values for prostate tumours is inconclusive.[59]

6.5 Recommendations

6.5.1 Research

As mentioned in Chapter 1, the biological effect of temporal factors during treatments has long been ignored and is recently coming to the fore. Therefore, research on this aspect of treatment still has to be undertaken. The results of the current study indicate that further research on conformal treatment techniques will be counterproductive. However, further research on the biologic effect of different dose-delivery patterns in

treatment techniques such as IMRT and cyberknife treatments may prove to be valuable.[2]

Research to determine the possibility of implementing these patterns of dose delivery to high-dose-rate brachytherapy may also prove to be a trivial endeavour for the radiologic reasons outlined in Section 5.3.2.

6.5.2. Beam sequencing protocol

The study provides evidence that it is unreasonable to change the current practice of sequencing multiple treatment beams in conformal treatment techniques because the benefit of the enhanced biologic effect is not evident.

6.6 Conclusions

The study determined that the radiosensitivity of low α/β ratio tumours was not altered by manipulating the sequence in which the fields are ordered during treatment. Although these results do not have any implications for the current practice, the results of Altman *et al* 's IMRT study[19] provide a template of how dose-delivery patterns could be used to aid treatment outcome positively, through either increasing cell kill in tumour tissue or enhancing normal tissue sparing. As a movement is being made internationally towards patient-specified treatment, including biological parameters and influences, further studies on temporal optimisation techniques are not only necessary but a requirement for treatment techniques with longer treatment times.