Monte Carlo Modelling of Tumour Growth, Hypoxia and Radiotherapy in Head and Neck Squamous Cell Carcinoma

Wendy M. Harriss

A report submitted for acceptance
as a thesis for the degree of
Doctor of Philosophy
from the School of Chemistry and Physics
University of Adelaide, South Australia, Australia

September 2011

Table of Contents

I. LIST OF FIGURES

II. LIST OF TABLES

ii) Oxic tumour Conventional Schedule simulation total cell kill results in terms of the number of 2 Gy fractions required to achieve total "basal" (stem, transit and level 1 differentiating cell) and stem cell only elimination, for accelerated repopulation (AR) onset times, and alpha beta ratios

APPENDIX C:

i) Moderately Hypoxic tumour simulation cell kill results for the total elimination of all stem transit and level 1 differentiating cells, for various onset times of accelerated repopulation (AR) and reoxygenation (ROx). Schedule numbers can be referred to in Tables 6.1 and 7.1 of this report.

ii) Oxic tumour simulation cell kill results for the total elimination of all stem transit and level 1 differentiating cells, for various onset times of accelerated repopulation (AR). Schedule numbers can be referred to in Tables 6.1 and 7.1 of this report.

III. ABREVIATIONS

AR	–	accelerated repopulation
BED	–	biological effective dose
CT	–	computed tomography
DNA	–	deoxyribonucleic acid
FHV	–	Fractional hypoxic volume
Gy	–	Gray = 1 Joule / Coulomb
HNSCC	–	head and neck squamous cell carcinoma
HP	–	hypoxic percentage (of cells in a tumour)
HYP-RT	–	'Hypoxic Radiotherapy' simulation model
IMRT	–	intensity modulated radiotherapy
KeV	–	kilo electron volt
KVp	–	kilo voltage potential
LET	–	linear energy transfer
LQ	–	linear quadratic (theory of cell survival)
M	–	molar
MV	–	mega voltage (x-ray beam)
MeV	–	mega electron volt
NTCP	–	normal tissue complication probability
OER	–	oxygen enhancement ratio
pO_2	–	partial pressure of oxygen
PTV	–	planning target volume
RBE	–	relative biological effectiveness
ROx	–	reoxygenation (during radiotherapy)
RT	–	radiotherapy
SCC	–	squamous cell carcinoma
SF_2	–	surviving fraction of cells after 2 Gy irradiation
TCP	–	tumour control probability
T_D	–	tumour double time
T_{pot}	–	potential tumour doubling time (equal to T_D when all cells are cycling clonogenic cells)

IV. ABSTRACT

Tumour hypoxia is the inadequate supply of oxygen in living tissue. Hypoxia is a major problem in the treatment cancer with ionising radiation because of the associated increase in radioresistance of hypoxic tumour cells. This effect can cause up to a three fold increase in the radiation dose required to kill the hypoxic cells compared to well oxygenated cells. Many locally advanced head and neck tumours exhibit hypoxia to some degree, and there is direct evidence that hypoxic tumour sub-volumes and their associated mean oxygenation levels have a direct influence on local tumour control after radiotherapy (Nordsmark 2005).

Currently, head and neck cancer radiotherapy local control rates lie at approximately 80% for early stage disease, but reduce significantly (often below 50%) for locally advanced tumours. Efforts to improve these statistics through dose and fractionation modifications in randomised clinical trials have been made in recent decades using alternate fractionation schedules, but the average prognosis has not improved significantly.

The effects of tumour reoxygenation during fractionated radiotherapy can assist in re-sensitising previously hypoxic tissue; however the complex dynamics and patient dependent characteristics of this phenomenon make the benefits difficult to quantify. Head and neck cancers, specifically head and neck squamous cell carcinoma (HNSCC), have also been shown to experience the phenomenon of accelerated repopulation during fractionated radiotherapy. Accelerated repopulation enhances cellular proliferation as a response to the trauma caused by treatment, and contributes to the low HNSCC local control rates after radiotherapy.

The modelling work developed for this report was undertaken to better understand the mechanisms and quantitative effects of HNSCC cellular kinetics and tumour oxygenation during growth and radiotherapy. The goal of individualising treatment planning for this disease was the motivation for developing the model. A key aim was to produce an end product to be used as an efficient and user-friendly radiobiological tool for the input on tumour specific properties such as tumour oxygenation and reoxygenation onset time, to investigate their effects on cell kill during radiotherapy.

To this end, a Monte Carlo model, named *HYP-RT (for **HYP**oxic-**R**adio**T**herapy simulation)*, was developed. *HYP-RT* simulates the tumour cell division process according to epithelial proliferative hierarchy, starting from a single stem cell. Monte Carlo methods were used to simulate the probabilistic nature of the biological and radiobiological mechanisms and parameters incorporated into the model, e.g. the distribution of cell cycle times (normal or exponential) and oxygenation levels (normal or log-normal), and the randomised methods of cell kill and oxygenation increase during treatment. Probabilistic methods were also used to make decisions during cell division, as to the type of daughter cell products that would emerge after the division of a mother cell.

After the growth of a 10^8 cell tumour, an algorithm was developed to model the effects of fractionated radiotherapy. This algorithm was designed to simulate the oxygen dependent radiosensitivity of individual tumour cells, as well as the effects of gradual reoxygenation and accelerated repopulation (through loss of stem cell division asymmetry). Both reoxygenation and accelerated repopulation could be onset at varying times after the start of treatment. Experimental animal work using HNSCC (FaDu cell line) xenografts was undertaken during this research, and showed that reoxygenation occurred very late in an accelerated radiation schedule (40 Gy in 2 weeks), indicating the need to investigate a range of reoxygenation onset times in the model (0 to 3 weeks).

Dynamic cell data was stored in a pre-allocated vector (the *Cellarray*) containing just over 10^8 object elements, with each element representing one tumour cell. This enabled efficient random access to the data. Linked list methods were used to chronologically order cells in the *Cellarray* based on their times of division. Model efficiency was paramount during model development, to ensure convenient use of the model for the current work and potential future research. Using linked list methods, the goal of a one hour maximum computation time to grow and treat a tumour was successfully achieved.

The model source code was written with the FORTRAN 95 programming language (complier v7.1.0, *Lahey Computer Systems* Inc.*)*, within the Visual Studio (2003, *Microsoft Corporation*) framework. Two additional graphical user interface programs were developed using the JAVA programming language (Java SE Development Kit 6.17), to 1) read in and interpret tumour data files, and 2) allow for the input of key tumour parameters before a simulation (or batches of simulations) and iteration over multiple parameter sets.

Cellular data and key algorithm parameters were written out to file at regular intervals (1000 hours by default), during tumour growth and before and after every dose fraction during treatment, for retrospective analysis. This data included the tumour pO_2 distribution, the instantaneous tumour growth rate and the number of cells of various types comprising the tumour.

Simulation results showed that tumour growth rate was strongly dependent on the percentage of stem cells in the tumour (modelled to be approximately 1% during growth). Incorporating a "moderately" hypoxic oxygen distribution increased tumour doubling times significantly, from 37 days for oxic tumours up to 65 days for moderately hypoxic tumours. This was attributed to the effects of oxygen dependent cell cycle slowing, cellular quiescence and necrosis.

Simulated conventional radiotherapy (5x2 Gy/wk) required on average an extra 16 Gy in total to achieve tumour control for moderately hypoxic compared to well oxygenated tumours. The effects of both accelerated repopulation and reoxygenation significantly altered the total doses required for tumour control, with accelerated repopulation effects dominating model outcomes. Accelerated repopulation and reoxygenation were found to be dependent on one another, making simulations of every combination of onset time for each effect necessary during model analysis.

During accelerated repopulation, a dose per fraction of 2.5 to 3.0 Gy was required to control the extra cell growth in an otherwise 2 Gy per fraction schedule. This equated to an extra 5 Gy being requiring to maintain tumour control for every week that the onset accelerated repopulation was brought toward the start of treatment. The benefits of reoxygenation reduced as the time of onset was delayed, with +1 Gy required to maintain tumour control for every week that reoxygenation was delayed.

Conventional fractionation simulation results had good agreement with standard Linear Quadratic theory, for the dose required to control well oxygenated tumours. However, comparison results were mixed for more complex cases involving hypoxic tumours with and without accelerated repopulation. When modelling altered fractionation schedules, simulation outcomes in terms of the total doses required for tumour control, agreed well with the prescriptions from published clinical trials. The most beneficial schedule, based on predicted total dose as well as biological effective doses (BED's) calculations for normal tissues, was the 10x1.1 Gy/week schedule (Pinto *et al.* 1991). However, there were up to 30 Gy differences in total dose and BED results when simulating

specific sets of tumour parameters for the same radiation schedule, highlighting the need for individualisation of treatment planning to improve the therapeutic ratio.

Four newly designed altered schedules were also simulated with the *HYP-RT* model. Results showed that using a concomitant boost at the beginning, rather than at the end of treatment, or using a "less aggressive" continuous hyper-accelerated radiotherapy (CHART) schedule (compared to the UK CHART schedule) may have potential therapeutic benefits compared to existing clinical schedules. Altering the oxygen enhancement ratio (OER) curve based on dose per fraction for the altered fractionation schedules, changed model results significantly for hyperfractionated schedules (up to 20 Gy). This highlighted the critical nature of the OER curve in predictive radiobiological tumour models.

In summary, the current research has involved the development, analysis and use of an efficient Monte Carlo tumour growth and radiotherapy model (*HYP-RT*). The model simulates a biologically plausible epithelial cell hierarchy, a large number of individual cells, tumour hypoxia, and the dynamics of reoxygenation and accelerated repopulation during radiotherapy. The user can input the desired oxygen distribution to describe the degree of tumour hypoxia as well as and the times of onset of treatment related effects, among many other cellular parameters. The model provides quantitative results regarding the total dose required to control a tumour, for a given fractionation schedule and tumour parameter set. It is hoped that computer models such as *HYP-RT* will be used in the near future as a tool to aid in the individualisation of radiotherapy planning, based on specific tumour experimental/imaging information, to improve prognosis for patients with HNSCC.

V. STATEMENT OF AUTHENTICITY

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Signed:……

Dated:……..

VI. ACKNOWLEDGEMENTS

I wish to give thanks to the School of Chemistry and Physics at the University of Adelaide, in accepting me into their postgraduate research program, and awarding me a scholarship which enabled me to study full time during the initial years of my PhD candidature. I also thank the University of Adelaide for further financial support which assisted in my attendance at several conferences and for the purchase of computer and experimental work equipment. Thank you to Dr Judith Pollard for your many years of dedicated work as the post-gradate Medical Physic program coordinator.

I am extremely grateful to have been accepted and welcomed into the Department of Medical Physics at the Royal Adelaide Hospital, and would like to whole-heartedly thank my supervisors Associate Professor Eva Bezak, Chief Medical Physicist, and Professor Eric Yeoh, Director of Radiation Oncology, for the their years of assistance, patience and support.

Eva, you have treated me with respect and kindness and have always given me your time when I have needed it, despite you very hectic schedule! I appreciate your efforts and consider working and studying with you an honour. I have very much enjoyed my time studying under your guidance, right from our first meeting at the beginning of my honours year in 2003.

Professor Yeoh, your support towards the Department of Medical Physics, and to post-gradate students is so very much appreciated. Thank you for your clinical guidance during my PhD research and for all of the wonderful editing work! I also appreciate your help in terms of financial support which assisted in funding my attendance at multiple conferences and workshops, as well as for these equipment needed for the animal experiments component of my research.

I would like to give kind thanks to Associate Professor Loredana Marcu, Senior Medical Physicist at the Royal Adelaide Hospital, for helping me to choose the direction of my research, for her kind support, and for her radiobiological knowledge which has assisted me greatly. Thanks also go to Professor Tim van Doorn and Dr Judith Pollard, for their supervision and guidance in the early years of this work.

Appreciation goes to all of the people that I have collaborated with, especially during the multi-disciplinary phase of animal experimental work, which was a new and exciting challenge for me. Thank you to Dr Tina Lavranos and Mrs Donna Fenu for their animal handling and cell line expertise, Dr Carlene Cullinane for the FaDu cell line donation, Professor Albert van der Kogel for the immunohistochemical imaging assistance, Mr Jim Manavis for tumour excise and cross-section advice and preparation, IMVS staff for animal handling and anaesthetic advice, Professor Cathy Malcontenti-Wison and Dr Peter Wong for *OxyLab* system assistance and equipment loan, and Mr Maarten Hermans, who joined the department as part of his masters work from the Technical University, Eidenhoven in the Netherlands, for his willing assistance and friendship during many hours of animal preparation and irradiation.

The Department of Medical Physics at the Royal Adelaide Hospital has been a welcoming and positive environment to study, with all team members showing me support and kindness during my candidature. Kind thanks go to the whole team and especially to Christine Robinson for her administrative assistance and caring friendship which has (and continues to) mean a great deal to me.

The love and support of my family, Lesley, John and Laura, my Grandparents, and my dear husband Dr Damien Phillips, have helped me immensely during my years of study. Thank you my family, your love has helped me to remain calm, focussed and remember what really matters in life. Damien, you have helped me more than I can put into words. You have assisted me emotionally and technically in achieving my goals for the model and getting to the finishing line of this long but extremely rewarding project. Thank you from the bottom of my heart for all that you do for me, for sharing your programming skills, for your patience, and most of all for your unconditional love.

VII. PUBLICATIONS & PRESENTATIONS

Publications in refereed journals

- Harriss-Phillips W. M., Bezak E., Yeoh K. (2011), *Monte Carlo radiotherapy simulations of accelerated repopulation and reoxygenation for hypoxic head and neck cancer.* Br J Radiol. **84** 903–918.
- Harriss W.M., Bezak E., Yeoh K., Hermans M. (2010), *Measurement of reoxygenation during fractionated radiotherapy in head and neck squamous cell carcinoma xenografts*. Australas Phys Eng Sci Med. **33** 251-63.
- Tuckwell W.M., Bezak E., Yeoh K., Marcu L.(2008), *Efficient Monte Carlo modelling of individual tumour cell propagation for hypoxic head and neck cancer*. Phys Med Biol. **53** 4489-507. (Note: published in my former name of W. M. Tuckwell).
  - ***Awarded the status of one of the top 20 articles in Phys Med Biol in 2008***

Published book chapters

- Harriss W. M. (2011), *Radiobiological modelling of tumour hypoxia and radiotherapy*. Recent Advances and Research Updates: Medical Physics. [ISSN 0972-4699] Chapter 16, 257-278.

Papers in preparation

- Harriss-Phillips W. M., Bezak E., Yeoh K. *Development of a radiobiological Monte Carlo model of hypoxic head and neck cancer and radiotherapy, HYP-RT.* To be submitted to Computational and Mathematical Methods in Medicine.

- Marcu L.M., Harriss-Phillips W.M., ***In silico*** *modelling of tumour response to treatment: current status.* To be submitted as a review paper to Computational and Mathematical Methods in Medicine.

Conference oral and poster presentations

International

- Harriss W.M., Bezak E., Yeoh K. (2009), *Computer and tumour xenograft modelling: dynamic hypoxia in head & neck radiotherapy*. Poster presentation. World Congress on Medical Physics and Biomedical Engineering. Munich, Germany.

- Tuckwell W.M., Bezak E., Yeoh K. (2008), *Monte Carlo Modelling of Hypoxic Head and Neck Squamous Cell Carcinoma and Radiotherapy*. Oral presentation. European Society for Therapeutic Radiology and Oncology Conference. Barcelona, Spain.

National

- Harriss-Phillips W.M., Bezak E., Yeoh K. (2011), *Observation of Hypoxia in HNSCC Xenografts during Fractionated Radiotherapy*. Oral presentation. Engineering and Physics Sciences in Medicine Conference. Darwin, Australia.
  - ***Awarded best Radiobiology proffered paper by Keynote speaker, Professor Wolfgang Dorr***

- Harriss-Phillips W.M., Bezak E., Yeoh K. (2011), *Simulations of Hypoxic HNSCC with Clinical Trial and New Radiotherapy Schedules using the HYP-RT model*. Oral presentation. Engineering and Physics Sciences in Medicine Conference. Darwin, Australia.

- Harriss W.M., Bezak E., Yeoh K. (2010), *Predicting individualised responses of hypoxic head and neck cancer to radiotherapy using Monte Carlo modelling.* Oral presentation. 3rd Modelling of Tumours "MOT" Meeting. Adelaide, Australia.

- Harriss W.M., Bezak E., Yeoh K. (2010), *Algorithm development and simulation outcomes for hypoxic head and neck cancer radiotherapy using a MC model: HYP-RT.* Oral presentation. Engineering and Physics Sciences in Medicine Conference. Melbourne, Australia.

- Harriss W.M., Bezak E., Yeoh K. (2009), *Computer and tumour xenograft modelling: dynamic hypoxia in head & neck radiotherapy*. Poster presentation. RANZCR, AIR, FRO, ACPSEM Combined Scientific Meeting. Brisbane, Australia.

- Harriss W.M., Bezak E., Yeoh K., Hermans M. (2009), *Computer and tumour xenograft modelling: dynamic hypoxia in head & neck radiotherapy*. Poster presentation. Engineering and Physics Sciences in Medicine Conference. Canberra, Australia.

- Harriss W.M., Bezak E., Yeoh K. (2008), *Monte Carlo modelling of head and neck cancer oxygenation.* Oral presentation. 2nd Modelling of Tumours "MOT" Meeting. Adelaide, Australia.

- Tuckwell W.M., Marcu L., Bezak E. (2006), *Individualising radiotherapy planning through Monte Carlo modelling of radiobiological principles for head and neck carcinoma*. Oral presentation. Engineering and Physics Sciences in Medicine Conference. Noosa, Australia.

- Tuckwell W.M., van Doorn T., Marcu L. (2005), *Temporal tumour modelling for individualised radiotherapy planning of head and neck cancer*. Oral presentation. Engineering and Physics Sciences in Medicine Conference. Noosa, Australia.

Other presentations

- Harriss W.M., Bezak E., Yeoh K. (2010), *Investigating the impact of reoxygenation in radiotherapy of hypoxic head and neck cancer using MC simulation and xenograft models*. Oral presentation. Royal Adelaide Hospital, *Medical Research Staff Prize Competition*. Adelaide, Australia.

- Harriss W.M., Bezak E., Yeoh K. (2010), *Predicting the responses of hypoxic head and neck cancer to radiotherapy using MC modelling.* Oral presentation (presented by E. Bezak). ACPSEM Queensland Branch Symposium. Brisbane, Australia.

- Harriss W.M., Marcu L., Bezak E. (2006), *Monte Carlo modelling of hypoxia and radiotherapy for head and neck cancer.* Oral presentation. University of Adelaide, Medical Physics and Biomedical Engineering *Postgraduate Student Paper Competition*. Adelaide, Australia.
  - Awarded first prize in the Medical Physics category

- Harriss W.M., van Doorn T., Marcu L. (2005), *Temporal tumour modelling for individualised radiotherapy planning of head and neck cancer*. Oral presentation. University of Adelaide, Medical Physics and Biomedical Engineering *Postgraduate Student Paper Competition*. Adelaide, Australia.
  - Awarded equal first prize in the Medical Physics category

- Harriss W.M., van Doorn T. (2004), *Increasing the efficiency of a temporal tumour growth model*. Oral presentation. University of Adelaide, Medical Physics and Biomedical Engineering Postgraduate Student Paper Competition. Adelaide, Australia.

For my family