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CANCER RADIOBIOLOGY

Dissertation submitted as partial fulfilment for higher diploma in radiobiology

BY

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DEDICATION

TO MY FAMILY

Acknowledgements

Great thanks to professor Jakob , the expert of the IAEA , for leading us on first steps in radiobiology.

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ABBREVIATIONS

RT	Radiotherapy
DNA	Deoxyribonuclic Acid
DSB	Double-Strand Break
pO_2	Oxygen Partial Pressure
G_2	Gap Two Phase (Cell Cycle)
G1	Gap One Phase (Cell Cycle)
S	DNA Synthesis (Cell Cycle)
M	Mitosis Phase (Cell Cycle)
LET	Linear Energy Transfer
IR	Ionizing Radiation
LDR	Low-Dose Rate
HDR	High-Dose Rate
TCP	Tumour Cure Probability
SSB	Single-Strand Break
NHEJ	Non-Homologous End Joining
OER	Oxygen Enhancement Ratio
HR	Homologous Recombination
RBE	Relative Biological Effectiveness
SOBP	Spread-Out Bragg Peak
ATM	Ataxia-Telangiectasia mutation
BRCA	Breast cancer susceptibility gene
CDK	Cyclin dependent kinase
CHK	Checkpoint Kinase 2
RAD	Retinoic acid receptor

Abstract

The work I have done in this dissertation, was mainly aimed at the literature review of radiotherapy radiobiology discussing the cure of tumours with ionizing radiation, from both the biological and physical point of view. The first chapter an introduction about the radiotherapy and includes: definition, working dose, benefit of radiotherapy, risk of radiotherapy, external and iternal radiotherapy and treatment planing.

In chapter two the theories of radiobiology and main effects caused by the radiation in the interaction with the biological matter were explained, the damages caused by the use of the low and high LET (linear energy transfer) particles to the mammalian cells were discussed. And discuss a therapeutic advantage may be gained by one of four hypothetical mechanisms: repair the damage of DNA, so when sublethal injury can be repaired if no further hits are sustained. Also the reoxygenation of the tumor is important for its effects on stabilization of free radicals produced by ionizing radiation. Hypoxic cells generally require an increased dose of radiation for lethal effect, redistribution, within the cell cycle depends on location of cells and their radiosensitivity also cells undergoing DNA synthesis, the S phase, are much more radioresistant than cells in other phases of the cell cycle, and repopulation of tumor cells is indecator of the surviving cells respond by increased regeneration or repopulation. Repopulation is a greater problem with rapidly proliferating tumors than slower growing neoplasms. These mechanisms are known as the classical four R's of radiation biology.

One of the important applications of radiobiology is the radiotherapy and cancer treatment , Experimental and theoretical studies in radiation biology contribute to the development of radiotherapy , in this dissertation we discussed the dose response relation so as the size of the tumor increases, and the dose needed for local control likewise increases, the risk of injury to normal tissue becomes greater becuse the idea of radiotherapy is to destroy the tumor cells without affect the normal cells, there the dose quantity and the time of rate is important factors for radiotherapy. But to safe the normal cells the dose fractionated, Conventional fractionation schedules are typically in increments of 1.8 to 2.0 Gy given five times per week for 6 to 8 weeks. Altered fractionation schedules have been developed in an attempt to optimize treatment results under various clinical circumstances, hyperfractionation is possible to increase the total dose, thereby increasing the probability of tumor control without increasing late complications.

In biological system the free radicals produced in water may react with essential macromolecules. A vast range of reaction takes place, most of which are unimportant for the survival and functioning of the cell. The most important reactions are those with DNA, because of the

uniqueness of many parts of this molecule. Damage of DNA by free radicals produced in water is called the *indirect effect* of radiation; ionization of atoms that are part of the DNA molecule is the *direct effect.*

The response of cells to ionizing radiation is strongly dependent upon oxygen, the enhancement of radiation damage by oxygen is dose-modifying, i.e. the radiation dose that gives a particular level of survival is reduced by the same factor at levels of survival. This allows us to calculate an oxygen enhancement ratio (OER), for the same level of biological effect. For most cells the OER for x-rays is around 3.0. However, some studies suggest that at radiation dose of 3 Gy or less the OER is actually reduced (Palcic and Skarsgard, 1984). This is an important finding because this is the dose range for clinical fractionation treatment.

The type of radiation used in radiotherapy depend on (LET), as LET increases, radiation produces more cell killing per Gy, These large differences in energy distribution, at the microscopic level and at equal absorbed dose, result in different biological effects dependant on the radiation quality. Some can be predicted: a higher RBE (ranging between 3 and >>5 in the conditions of fast neutron therapy), different shapes of the dose-effect relationships for tumours and normal tissues and hence the possibility to obtain a differential effect and a therapeutic benefit.

So to know how cancer treated with radiation, we must calculate and know the OER, RBE, repairing of DNA and how to fractionate the dose and what type of radiation must be used, and what a relation between all these variables.

Chapter One

1. Introduction

Radiotherapy uses carefully measured doses of radiation to treat cancer. The type of radiotherapy most people have used beams of high-energy rays, usually x-rays. The radiotherapy machine aims specific amounts of the radiation only to the area of the body that it is pointed at and nowhere else.

A high dose of radiation damages cells and stops them from growing and dividing. Cancer cells, which are abnormal cells, do not recover. Normal cells that are affected usually recover or repair themselves quite quickly. Any side effects, which occur during treatment, are usually temporary.

The purpose of radiotherapy is to destroy the cancer cells while causing as little damage as possible to normal cells. It can be used to treat many kinds of cancer in almost any part of the body. Curative treatment, which is sometimes called radical treatment, aims to give long-term benefits to people. Sometimes radiotherapy is given on its own or it may be given alongside other treatment. Radiotherapy may be given before surgery to shrink a tumour or after surgery to stop the growth of cancer cells that may remain. It can also be given before, during, or after chemotherapy (anti-cancer drugs) to improve treatment results (www.royalmarsden.nhs).

Palliative treatment aims to shrink tumours and reduce pain or relieve other cancer symptoms. While a cure is not likely palliative radiotherapy may also prolong life.

Radiotherapy can damage or destroy normal cells as well as destroying cancer cells and cause treatment side effects. Most side effects are temporary. Any side effects, which occur during treatment, are usually temporary. There may be a small risk of long term, or late, permanent effects from radiotherapy. However, side effects are rarely severe. ladie should not become pregnant before or during radiotherapy because radiotherapy may injure the foetus, especially in the first three months of a pregnancy.

Radiation therapy can be given in one of two ways; external or internal.

External radiotherapy

External radiotherapy is usually given as a course of several treatments over days or weeks while internal treatment may only happen once or a small number of times.

External radiotherapy is usually given during outpatient visits to a hospital cancer centre. A machine directs the high-energy rays, usually x-rays, at the cancer site and a small area of normal tissue surrounding it. Patient is positioned carefully on a treatment couch and then the machine will be directed exactly at the area to be treated, often from different angles. Treatment takes several minutes and is painless. Before the start of radiotherapy patient usually need to attend the hospital for treatment planning. External radiotherapy doesn't make the patient radioactive and he or she can safely mix with other people, including children, at anytime (www.royalmarsden.nhs).

Internal radiotherapy

Brachytherapy is treatment, in which solid radioactive sources are placed inside a body cavity or needles are placed in the tumour. This usually involves staying in hospital for a few days until the radioactive source has been removed.

Another type of internal radiotherapy involves using a liquid source of radiation and is called radionuclide (or radioisotope or unsealed source therapy). It can either be taken by mouth or given as an injection into a vein. For this type of treatment, the patient needs to stay in hospital for a few days until most of the radioactivity has disappeared from his body. Occasionally, with radioactive treatment or with treatment with radioactive 'seeds', the patient will be made radioactive for a few days. You may then have some temporary restrictions on your social life (www.royalmarsden.nhs).

Chapter Two

2. Radiobiology

2.1 Introduction:

The energy of therapeutic radiation is high enough to eject an electron from a target molecule, thus, the term ionizing irradiation. Ionizing energy is distributed randomly within the cell so that the x-rays hit a wide array of molecules. A DNA double strand break is generally believed to be responsible for cell death, which is determined by cells that are no longer able to undergo cell division. The injury responsible for cell death can occur directly or indirectly, via free radicals (molecules with an unpaired electron, e.g., DNA → DNA•). Free radicals are highly reactive and can either be reduced by cellular mechanisms (repaired DNA) or stabilized by oxygen (permanent DNA-OO• damage). Inadequate repair of DNA lesions, either nucleotide base damage or single/double strand breaks, can lead to cell death or mutation (A Boriano and Cperoni; 2005).

Radiation oncologists and researchers have recognized the serious nature of complications from radiation exposure. Late radiation adverse sequelae is a continuum of acute, subacute, and long-term normal tissue effects that have been observed in those receiving therapeutic radiation for cancer, and survivors of nuclear accidents, occupational exposure, and exposure from the atomic bomb (Y Chen *et al*; 2006).

Research on biomarkers for radiation injury has vital revealed information to the understanding of molecular targets and cellular pathways of radiation, starting with initial injuries and continuing through the perpetual normal tissue late effects. Not only do biomarkers reveal mechanisms of radiation injuries, but they may also serve as potential tools for dose estimation (i.e., biodosimetry), which is critical for managing acute radiation damage by facilitating the identification of those who received low-, moderate-, or high-dose exposure, thereby providing complementary measures in predicting potential long-term sequelae of radiation accidents or of cancer treatment (Y Chen *et al*; 2006).

In 2003, an estimated 1.3 million new cases of cancer were diagnosed in the United States. Many of these patients received some form of radiotherapy (RT) as part of their treatment. After surgery, RT is arguably the most important treatment for cancer, especially for localized disease that has not spread. Ionizing radiation is used to treat virtually all types of solid malignancies, but to varying degrees of success. That is, some tumors are highly responsive to low doses of radiation (e.g., lymphomas, seminomas), and other tumors are typically very radioresistant and tend to progress even after high radiation doses (e.g., melanoma, glioblastoma). A further difficulty is that treatment fails in a considerable number of patients treated with ionizing radiation with curative intent not only because of distant metastatic spread, but also because of local treatment site failure. The reasons for such local RT failure are multiple and varied. Tumor

factors, such as location, size, and inadequate vascular supply (hypoxia), can all play a role in the lack of responsiveness of neoplasms to ionizing radiation. Perhaps most important, however, are the cellular and genetic factors, such as differential tissue-specific gene expression (e.g., p53, ataxia telangiectasia mutated [ATM] status), that may result in radiation-resistant cellular phenotypes. Support for the role of differential gene expression in determining radiation sensitivity comes in part from observations that cells from the same tissue of origin, but from different patients, can show varying radiation sensitivities. That is, tumors from different patients with the same histologic diagnosis can show varied responses to ionizing radiation. Such differential radiosensitivity can also be present within a single tumor. This was the observation of Weichselbaum, who reported that four cell lines clonally derived from the same tumor source showed different radiation sensitivities (Timothy M. Pawlik, *et al*; 2004).

2.2 Random Cell Death

The deposition of ionizing energy is a random event which leads to radiochemical injury. Therefore, any given cell within a tumor has an equal chance of being hit by a given dose so that the same proportion of cells within the tumor is damaged per dose. In other words, the same dose of radiation will reduce the cell population from 100 cells to 10 cells as it will 10 billion cells to 1 billion cells. This means that a more radiation is needed to eradicate larger tumors. Furthermore, a tumor is no longer palpable when it is reduced to 10^5 cells, so clinical response rates do not relate to effectiveness of the radiation dose. Finally, this random nature of cell death applies to normal tissue as well as tumor cells. A therapeutic advantage may be gained by one of four hypothetical mechanisms. These mechanisms are known as the classical four R's of radiation biology (A Boriano and C Peroni ; 2005).

2.2.1 Repair of sublethal injury

When a secondary electron passes through matter, a cell may be exposed to either dense or sparse ionization. It is thought that cells are more likely to repair damage inflicted by sparse ionization within the field of radiation. This sublethal injury can be repaired if no further hits are sustained. Therefore, a greater total dose is needed to produce a biologic effect when given in several fractions as opposed to a single fraction. The greater the number of fractions, the greater is the opportunity for repair between dose fractions. However, the same biologic effect requires a greater total dose. In most tissues, sublethal injury is repaired within 3 hours, but up to 24 hours may be necessary for some tissues. This concept explains why radiation therapy is fractionated. It allows repair of injured normal tissue, providing an overall therapeutic advantage over tumor cells. In contrast, this also may explain the radioresistance of certain malignant cell types, which have a remarkable ability to repair sublethal injury (i.e., melanoma).

Exposure to ionising radiation creates lesions within cellular DNA that cause a range of responses, including cell-cycle arrest, apoptosis, reproductive death and senescence. About 40 DNA double-strand breaks (DSBs) are induced in a cell for each Gy of absorbed dose, and these are thought to be the lesions responsible for radiation-induced cell death, with a single unrepaired DSB sufficient to elicit this response.

Endogenous free-radical attack or DSB introduced during DNA replication induces a response that is largely conserved from yeast to man. Nearly all extremely radiosensitive experimental cell lines carry mutations in genes regulating the response to DSB, and when cells are given a normal copy of the mutated gene, radiation sensitivity is corrected. The importance of DSB repair is readily understandable, because, unless every DSB is repaired before cell division, chromosomes will not segregate normally at mitosis. Several features of DSBs make them difficult to repair compared with single-strand breaks and other forms of genetic damage. As both strands of the DNA helix are broken, the broken ends can physically dissociate. Separation of ends predisposes to interactions with breaks at other sites that lead to translocations and deletions. Furthermore, the ends of the break have usually sustained damage to bases that need to be removed and replaced. Although bases damaged at a single-strand break can be replaced using the opposite complementary strand as a template, this opposing template has also been damaged at a DSB and is unavailable (Tutt & Yarnold; 2005).

2.2.2 Reoxygenation of Tumors

Oxygen is important for its effects on stabilization of free radicals produced by ionizing radiation. Hypoxic cells generally require an increased dose of radiation for lethal effect. Hypoxic regions within cancerous tissue can occur secondary to temporary constriction or collapse of capillaries or when tumors outgrow their blood supply. During radiation treatment hypoxic areas within the tumor decrease as the size of the tumor diminishes, compressed blood vessels open, and hypoxic cells are brought closer to capillaries. Reoxygenation is another reason why radiation is given in fractionated doses. Tumor hypoxia is another potential cause of radioresistance. Recently, hypoxic cell radiosensitizers and agents selectively toxic to hypoxic cells have been developed for clinical use with concurrent radiotherapy.

As an alternative to radiotherapy regimens regarded as "conventional" (fractionated doses of 1.8 – 2.2 Gy given once daily, 5 d/wk for about 7 weeks), altered fractionation schedules using multiple daily radiation fractions have been proposed as a promising strategy to treat rapidly proliferating tumors. These protocols include hyperfractionation and accelerated fractionation. Generally, a therapeutic gain for these regimens is expected for the patient as long as acute reactions remain tolerable and later effects are unchanged. The aim of accelerated fractionation is to minimize the potential for tumor growth or regeneration during therapy. Moreover, the therapeutic gain could

exploit the sensitization due to the cell cycle redistribution. The dynamic changes in the tumor microenvironment have been largely ignored as factors that may contribute to greater radiosensitivity of tumors in the protocols that use multiple daily fractions. This is very different from the classic "reoxygenation effect" described for conventional radiotherapy regimens. A large amount of evidence has shown that the reoxygenation of tumors occurs 24 –72 h after irradiation. It has been shown that this late reoxygenation contributes significantly to the efficacy of a second irradiation. The molecular basis of reoxygenation at 24 h was recently investigated. It turns out to be mediated by nitric oxide, following changes in expression and activity of endothelial nitric oxide synthase (eNOS) and caveolin, with a consequent increase in blood flow and oxygen delivery. In contrast to the "late" reoxygenation effect, which occurs 1 or 2 days after irradiation, little is known about the changes in the microenvironment at early stages just after irradiation. In pioneering studies, Kallman and Dorie suggested that the phenomenon of reoxygenation could be caused by the reacquisition of radiosensitivity by those cells that are able to survive irradiation because they were hypoxic at exposure. They described a rather rapid phenomenon in several tumor lines. The indirect evidence of reoxygenation was based on the determination of the hypoxic fraction using dose-survival curves of suspended cells taken from irradiated tumors. They found that the hypoxic fraction had already declined 1 h after a single radiation fraction of 10 Gy. Later, the development of oximetry technologies allowed direct *in vivo* measurements of oxygen partial pressure (pO2). Using 19F-nuclear magnetic resonance relaxometry, pO2 measurements suggested a reoxygenation at 1, 4, and 10 h after irradiation of 20 Gy. The only study that has measured pO2 early after irradiation using a clinically relevant dose (2 Gy) was by Weissfloch *et al.* with the Eppendorf system. They showed an increase in tumor oxygenation 2 h after irradiation and a decrease at 24 h after irradiation. No study has described a continuous measurement of tumor oxygenation to determine the time sequence and extent of reoxygenation in tumors early after irradiation(N Crokart; 2005).

2.2.3 Redistribution within the Cell Cycle

Each individual cell's position in the cell cycle influences its radiosensitivity. Cells undergoing DNA synthesis, the S phase, are much more radioresistant than are cells in other phases of the cell cycle. There is increasing evidence that the ability of a cell to be delayed in the G_2 phase of the cell cycle corresponds to its ability to survive irradiation. Studies of the RAD9 gene mutation producing radiosensitivity in yeast have shown these cells do not undergo a delay in G_2 following irradiation. In addition, radioresistant rat embryo cells transformed with oncogenes, H-ras and c-myc, showed a G_2 delay and more radioresistance than control cells. When radiation treatment is fractionated, surviving cells redistribute into more sensitive phases of the cell cycle, making them more susceptible to subsequent fractions. The sensitizing effect of redistribution tends to offset

sublethal injury repair. Furthermore, rapid cycling cells redistribute better between fractions than slowly cycling cells. Skin and mucosa cells cycle rapidly and are responsible for acute reactions to irradiation. Connective tissue, brain and blood vessels cycle more slowly and are responsible for late effects. It is the tissues responsible for late complications that are spared more by fractionation of treatment.

Multiple pathways are involved in maintaining the genetic integrity of a cell after its exposure to ionizing radiation. Although repair mechanisms such as homologous recombination and nonhomologous end-joining are important mammalian responses to double-strand DNA damage, cell cycle regulation is perhaps the most important determinant of ionizing radiation sensitivity. A common cellular response to DNA-damaging agents is the activation of cell cycle checkpoints. The DNA damage induced by ionizing radiation initiates signals that can ultimately activate either temporary checkpoints that permit time for genetic repair or irreversible growth arrest that results in cell death (necrosis or apoptosis). Such checkpoint activation constitutes an integrated response that involves sensor (RAD, BRCA, NBS1), transducer (ATM, CHK), and effector (p53, p21, CDK) genes. One of the key proteins in the checkpoint pathways is the tumor suppressor gene p53, which coordinates DNA repair with cell cycle progression and apoptosis. Specifically, in addition to other mediators of the checkpoint response (CHK kinases, p21), p53 mediates the two major DNA damage-dependent cellular checkpoints, one at the G1–S transition and the other at the G2–M transition, although the influence on the former process is more direct and significant. The cell cycle phase also determines a cell's relative radiosensitivity, with cells being most radiosensitive in the G2-M phase, less sensitive in the G1 phase, and least sensitive during the latter part of the S phase. This understanding has, therefore, led to the realization that one way in which chemotherapy and fractionated radiotherapy may work better is by partial synchronization of cells in the most radiosensitive phase of the cell cycle (T M. Pawlik; 2004).

2.2.4 Repopulation

As cells are lost to radiation injury and death within a given population of normal or tumor cells, the surviving cells respond by increased regeneration or repopulation. Repopulation is a greater problem with rapidly proliferating tumors than slower growing neoplasms. Regeneration is therefore one of the determinants for planning the length and timing of a course of therapy, and a balance between adequate tumor control and sufficient sparing of acutely reacting normal tissues to allow recuperation must be reached. Accelerated treatment schedules with twice-daily fractionation and combined accelerated-hyperfractionated schedules have been developed to diminish the opportunity for tumor repopulation. It is likewise, the reason not to delay treatment after incomplete resection and to avoid protracted courses of therapy or split-course treatment schedules(A Boriano and C Peroni ; 2005).

2.3 The time-scale of effects in radiation biology

Irradiation of any biological system generates a succession of processes that differ enormously in time scale. This is illustrated in Figure.1 where these processes are divided into three phases.

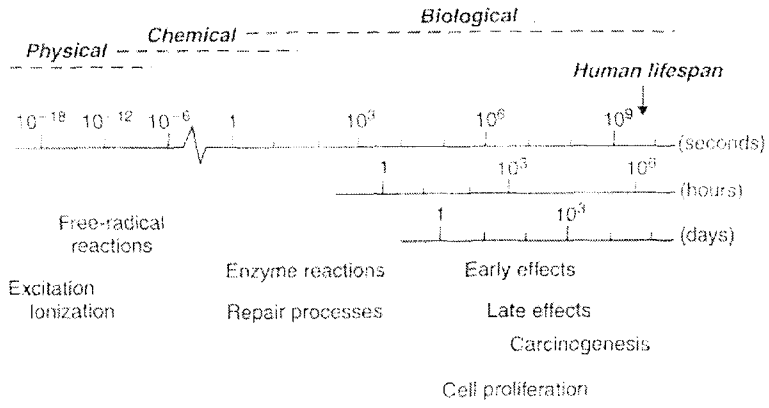


Figure.1: Time scale of the effects of radiation exposure on biological systems.

The physical phase consists of the interaction between charged particles and the atoms of which the tissue is composed. High speed electron takes about 10^{-18} seconds to traverse the DNA molecules and about 10^{-14} seconds to pass across a mammalian cell. As it does so, it interacts mainly with the orbital electrons, ejecting some of them from atoms (ionization) and raising other to higher energy levels within an atoms or molecule (excitation). If sufficiently energetic, these secondary electrons may excite or ionize other atoms near which they pass, giving rise to cascade of ionizing events. For 1 Gy of absorbed radiation dose there are in excess of 10^{5} ionizations within the volume of any cell of diameter of 10μm.

The chemical phase describes the period in which the damaged atoms and molecules react with other cellular components in rapid chemical reactions. Ionization and excitation lead to the breakage of chemical bonds and the formation of broken molecules, known as 'free radical'. These are highly reactive and engage in a succession of reactions that lead eventually to the restoration of electronic charge equilibrium. Free-radical reactions take place within approximately partial second of radiation exposure. An important characteristic of the chemical phase is the compensation between scavenging reactions, for instance with sulphydryl compounds that inactivate the free radical, and fixation reaction that lead to stable chemical changes in important biological molecules.

The biological phase includes all subsequent processes. These begin with enzymatic reactions that act on the residual chemical damage. The vast majority of lesions, for instance in DNA, are

successfully repaired. Some rare lesions fail to repair and this what leads eventually to cell death. Cells take time to die; indeed, after small dose of radiation they may undergo a number of mitotic divisions before dying. It is the killing of stem cells and the subsequent loss of the cells that they would have given rise to, that causes the early manifestations of normal-tissue damage during the first week and month after irradiation exposure. Examples are: breakdown of the skin or mucosa, denudation of the intestine and haemopoietic damage. A secondary effect of cell killing is compensatory cell proliferation, which occurs both in normal tissue and tumours. At later times after the irradiation of normal tissue the so called 'late reactions' appear. These include fibrosis and telangiectasia of the skin, spinal-cord damage and blood vessel damage. An even later manifestation of radiation damage is the appearance of secondary tumours (i.e. radiation carcinogesis). The time-scale of the observable effects of ionizing radiation may thus extend up to many years after exposure (A Boriano and C Peroni; 2005).

Chapter Three

3. Cancer Treatment

3.1 Introduction:

Experimental and theoretical studies in radiation biology contribute to the development of radiotherapy at three different levels, moving in turn from the most general to the most specific:

Ideas: providing a conceptual base for radiotherapy, identifying the mechanisms and processes that underline the response of tumour and normal tissues to irradiation and which help to explain the observed phenomena. Examples are: hypoxia, reoxygenation, tumour cell repopulation or mechanism of repair of DNA damage.

Treatment strategy: development of specific new approaches in radiotherapy. Examples are hypoxic cell sensitizers, high LET-radiotherapy and hyperfractionation.

Protocols: advice on the choice of schedules for clinical radiotherapy, for instance conversion formulae for changes in fractionation or dose rate, or advice on whether to use chemotherapy concurrently or sequentially with radiation. We may also include under this heading methods for predicting the best treatment for the individual patient (individualized radiotherapy).

There is no doubt that radiobiology has been very fruitful in the generation of new ideas and in the identification of potentially exploitable mechanisms. A variety of new treatment strategies have been produced, but few of these have so far led to demonstrable clinical gains(A Boriano and C Peroni; 2005).

3.2 Radiotherapy

Radiotherapy is one of the two most effective treatments for cancer. Surgery, which ofcourse has the longer history, is in many tumour types the primary form of treatment and it leads to good therapeutic results in a range of early non-metastatic tumours. The combination between radiotherapy and surgery often achieve a reasonable probability of control for many tumours, and in case of tumour of head and neck, cervix, bladder, prostate and skin the only radiotherapy gives good results. In addition to these examples of the curative role of the radiation therapy, many patients gain valuable palliation by radiation. Chemotherapy is the third most important treatment modality at present time. Many patients receive chemotherapy at some point in their management and useful symptom relief and diseases arrest are often obtained.

The following is a brief outline of the role of radiotherapy in six disease sites:

Bladder: the success of surgery or radiotherapy varies widely with the stage of disease; both approaches give 5-year survival in excess of 50%.

Breast: early breast cancer, not known to have metastasized, are usually treated by surgery and this have a tumour control rate in the region of 50-70%. Radiotherapy given to the chest wall and regional lymph nodes increases control by up to 20%. Hormonal therapy and chemotherapy also

have significant impact on patient survival. In patients who have evidence of metastatic spread at the time of diagnosis, the outlook is poor.

Cervix: disease that has developed beyond the *in situ* stage is often treated by combination of intracavitary and external-beam radiotherapy. The control rate varies widely with stage of the disease, from around 70% in stage I to perhaps 7% in stage IV.

Lung: most lung tumours are inoperable and for them the 5-year survival rate for radiotherapy combined with chemotherapy is in the region of 5%.

Lymphoma: in Hodgkin's disease radiotherapy alone achieves a control rate of around 50% and when combined with chemotherapy this may rise to 80%.

Prostate: where there is evidence of local invasion, surgery and radiotherapy have similar level of effectiveness, with 10-years control rates in the region of 50%. Chemotherapy makes a limited contribution to local control.

Very substantial number of patients with common cancers achieves long-term tumour control largely by the use of radiation therapy. There are three main ways in which an improvement in radiotherapy might be obtained (A Boriano and C Peroni; 2005):

- rising the standard of radiation dose prescription and delivery with respect to those currently in use.
- improving radiation dose distributions beyond those that are conventionally achieved, either using techniques of conformal radiotherapy with photons, or use of hadronic beams.
- exploring radiobiological initiatives.

3.2.1 Dose-Response Relations

The probability of controlling cancerous lesions with radiotherapy depends on the size of the tumor and the dose of radiation given. The dose-response relation for small, well-vascularized neoplasms is steep, because they are relatively homogeneous, are well oxygenated and have approximately the same number of cells. Bulky tumors, however, are more heterogeneous with considerable variability in number of cells and oxygenation. Therefore, the dose-response curve is much shallower. The dose-response relation for normal tissue injury is the limiting factor in the amount of irradiation that can be given. As the size of the tumor increases, and the dose needed for local control likewise increases, the risk of injury to normal tissue becomes greater.

3.2.2 Radiotherapy Fractionation

Conventional fractionation schedules are typically in increments of 1.8 to 2.0 Gy given five times per week for 6 to 8 weeks. Altered fractionation schedules have been developed in an attempt to optimize treatment results under various clinical circumstances. In essence, the objective of altered fractionation is to improve the therapeutic ratio through an alteration of time, dose, and/or fractionation based on the differential response of tumors and normal tissues to these altered

schedules. Accelerated fractionation involves two or more dose fractions of the conventional size per day in an attempt to shorten overall treatment time. In theory, this may minimize tumor repopulation during treatment and, therefore, increase the probability of tumor control for the same total dose. Hyperfractionation involves the administration of two or smaller dose fractions per day for a conventional or slightly longer treatment time. Theoretically, with hyperfractionation it is possible to increase the total dose, thereby increasing the probability of tumor control without increasing late complications (A Boriano and C Peroni; 2005).

In normal human cells, the DNA damage that results from exposure to ionizing radiation (IR) activates cellular responses that include cell cycle arrest, DNA damage repair, and apoptosis. The responses to genotoxic stress are controlled by cell cycle checkpoints, the biochemical signaling pathways that induce cell cycle delays, coordinate DNA damage repair, maintain cell cycle arrest to complete the repair, and then reinitiate cell cycle progression. lthough some DNA damage checkpoint cascades respond to DNA damage in quiescent cells, most operate only in proliferating cells, with the nature of the response dependent on variables such as cell type and differentiation, extent of DNA damage, and the position of the cell in the cell cycle. Thus normal cells that have not passed the G1 restriction point will arrest in G1, S-phase cells will undergo a delay in S and may undergo a subsequent G2 arrest, and hose cells already in G2 will be prevented from entering M-phase until repair of potentially lethal damage is complete. The use of fractionated radiation therapy, repeated irradiation with equal IR doses at approximately 24-h intervals, is based in part on the assumed differences in the responses that normal and tumor cells manifest to IR-induced DNA damage. Additionally, the combination of the three "Rs" of radiobiology (repair, reassortment, and repopulation) greatly influences the effectiveness of a regimen such as this. The underlying assumptions are that in normal cells sublethal IR-induced DNA damage is completely repaired provided that the time between doses is sufficient for the repair of the previous damage and that damage repair in normal cells is greater than in tumor cells. Thus, for tumor cell lines, exposures to subsequent doses of IR should result in cell killing equal to the first fraction and tumor cells should have more nearly exponential killing on these repeated exposures compared with normal cells. Accordingly, fractionated-dose radiotherapy is delivered under the working premise that radiosensitivity does not change with each subsequent fraction and therefore, decreases in cell survival following the re-exposures are exponential. The assumption of equal cell killing per fraction has been investigated in studies using both normal and tumor cell lines. Although the results have varied, they do suggest that this basic assumption may not be valid and thus this issue is controversial (A. Tutt and J. Yarnoldy; 2005).

In a recent publication, it was claimed that the belief that low-dose rate (LDR) must be inherently superior radiobiologically to high-dose rate (HDR) is wrong if late-responding normal tissues all

do have much longer repair half-times than tumors. Although a long repair half-time would limit the repair during an LDR course, it would not affect HDR treatments where there is no repair during, and full repair between, fractions. Long repair half-times of 4.4 h (3.8–4.9 h) for s.c. fibrosis and 3.8 h (2.5– 4.6 h) for skin telangiectasia were estimated in an analysis from the Continuous Hyperfractionated Accelerated Radiotherapy (CHART) trial, in which head-and-neck cancer patients were treated with three fractions per day spaced 6 h apart.Those repair half-times are in agreement with indications from hyperfractionation protocols for the existence of long repair half-times in late-responding normal human tissues (e.g., 3). Using the assumption that repair half-times for normal tissue cells at risk in cervical cancer brachytherapy are as long as those observed for tissues reported in the CHART analysis, i.e., 2.5 h or even 4 h, and considering complete repair between multiple HDR fractions, it was shown that HDR with fraction sizes of 5–7 Gy should be superior to LDR at a dose rate of 0.5 Gy/h. Added to the benefit of geometrical sparing, positive clinical results of HDR treatment of cervical carcinoma with 5–7 Gy per fraction (1) might be explained on the basis of such differences in repair half-times between normal tissue and tumor (P Sminia; 1998).

Fractionation of HDR requires selection of the number of fractions or fraction size. In clinical practice, often the choice is made of lowering the fraction size to reduce the biologic dose ratio of late-responding normal tissue to tumor. To overcome a too-large difference in overall treatment time between LDR and fractionated HDR that would allow tumor cells to repopulate, HDR regimens with multiple fractions per day are being used. Of great concern, however, is the incompatibility of tissue repair kinetics with the time interval between fractions. Repair may be compromised if the repair half-time of the tissue exposed is on the same order of magnitude as the time interval between the fractions. "This could negate any benefit achieved by the use of low dose per fraction, and it could well be better to use fewer (larger) treatments with longer time between fractions" (A Boriano and C Peroni ; 2005).

They demonstrate that hypofractionation in HDR might have its opportunities for widening the therapeutic window, but definitely has its limits. For each specific combination of the parameters, a theoretical optimal HDR fraction size with regard to *relative* or *absolute* normal tissue sparing can be estimated, but because of uncertainty in the biologic parameters, these hypofractionation schemes cannot be generalized for all HDR brachytherapy indications (Peter Sminia, *et al*, 1998).

Because of the large amount of energy deposited in the critical cellular target by a single high-LET particle track, the repair phenomena are less. High-LET radiation is thus particularly efficient against cancer cells that have a high capability for repair, hence in principle for prostate tumours that have a very low α/β ratio.

Unfortunately, the sparing of late normal tissue reactions by low dose fractions, the feature that underpins the success of hyperfractionated photon therapy is reduced in the case of high-LET

therapy. There is also less long term repair and more residual injury in normal tissues after high-LET irradiation.

One feature of high-LET therapy is that the reduced influence of dose per fraction leaves more flexibility for selecting the fractionation scheme, and it was suggested as reasonable for neutrons to shorten the overall time and to deliver the total dose tolerated by the relevant "late-effect" tissues(s) in the shortest time consistent with acceptable acute response (A Wambersie *et al*; 2003).

3.3 Response curve of radiotherapy:

The effects of radiation exposure become apparent during the weeks, month and years after radiotherapy. These effects are seen both in tumour tissues and normal tissues that surround a tumour and which are unavoidably exposed to radiation. The response of a tumour is seen by *regression*, often followed by *regrowth*, but perhaps with failure to regrow during the normal lifespan of the patient (which is termed *cure* or *local control*).

The response of normal tissue to therapeutic radiation exposure range from those that cause mild discomfort to other that are life threatening. The speed at which a response develops varies widely from one tissue to another and often depends on the dose of radiation that the tissue receives. Generally speaking the haemopoietic and epithelial tissues manifest radiation damage within weeks of radiation exposure, whereas damage to connective tissue becomes important at later times.

The improvements in cancer curability, long-term survival, and organ preservation after radiation treatment, as well as improvements in combined modality therapy have resulted in many cancer patients living with the late, adverse effects. Long-term follow-up of these individuals reveals progressive fibrosis and functional impairment of organs along with associated comorbidities such as decreased tissue compliance, accelerated senescence of organs, the decline of endocrine function, infertility, cardiac dysfunction, joint stiffness, myelopathy, neurocognitive impairment, and others. Apart from the tissue and organ structural damage, radiation DNA damage may cause malignant transformation of normal cells in the form of mutagenesis and carcinogenesis. Both hematopoietic malignancy and solid tumors have been observed. Although a relatively rare event, radiation-induced cancer has been reported in survivors of cancer therapy, as well as in survivors of atomic radiation from atomic bombing, occupational exposure, or nuclear accidents. Second malignancy was also reported in individuals who received radiation for benign conditions such as thymic gland enlargement in infants, ankylosing spondylitis of spine, tinea capitis in children, and others (Y Chen *et al*; 2006).

The damage that is observed in an irradiated tissue increases, reaches a peak, and then may decline (Figure2A). It could be possible use the measured response at some chosen time after

irradiation, such at the time of maximum response, but the timing of the peak may change with radiation dose and this would lead to some uncertainty in the interpretation of the results. A common method is to calculate the cumulative response by integrating this curve from left to right (Figure2B). The response for some normal tissue gives a cumulative curve that rises to a plateau, and the height of the plateau is a good measure of the total effect of that radiation dose on the tissue. Other normal tissue response, in particular the late responses seen in connective and vascular tissues, are progressive and the cumulative response continues to rise.

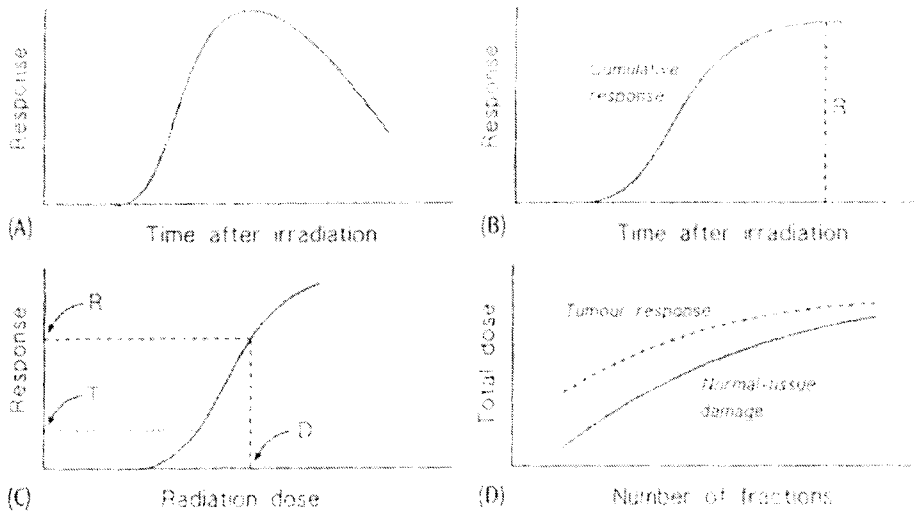


Figure2: Four types of chart leading to the construction of an isoeffect plot. (A) Time-course of radiation damage in a normal tissue. (B) The cumulative response. (C) A dose-response relationship, constructed by measuring the response (R) for various radiation doses (D). (D) Isoeffect plot for a fixed level of normal tissue damage.

The next stage in a study of the radiation response of a tissue consist in varying the radiation dose and thus investigating the dose-response relationship (Figure2C). Radiation dose-response curves have a sigmoid shape, with the incidence of radiation tending to zero as dose goes to zero and tending to 100% at very large doses. Many mathematical functions could be used with these properties, but the most standard formulation used is the Poisson distribution. Munro and Gilbert published a landmark paper in 1961 in which they formulated the target cells hypothesis of tumour control: 'The object of treating a tumour by radiotherapy is to damage every single potentially cell to such an extent that it cannot continue to proliferate'. From this idea and the random nature of cell killing by radiation they derived a mathematical formula for the probability of the tumour cure after irradiation 'of a number of tumours each composed of N identical cells'. More precisely, they showed that this probability depends only on the average number of clonogens surviving per tumour. When describing tumour cure probability (TCP), it is the

probability of zero surviving clonogens in a tumour that is of interest. This is the zero-order term of the Poisson distribution and if λ denotes the average number of clonogens after irradiation this is simply:

$$TPC = e^{-\lambda} \qquad (1.1)$$

The simple exponential was later replaced by the linear-quadratic model and thus we arrived at what could be called the standard model of tumour control:

$$TCP=\exp[-N_0*\exp(-\alpha D-\beta dD)] \qquad (1.2)$$

Here N_0 is the number of clonogens per tumour before irradiation and the second exponential is simply the surviving fraction after dose D given with dose per fraction *d*, according to the linear quadratic model. Thus when we multiply these two quantities we obtain the average number of surviving clonogens.

Diagrams similar to Figure2A, B, C can also be constructed for fractionated radiation treatment, although the results are easiest to interpret when the fraction are given over a time that is short compared with the time scale of development of the response. If we change the schedules of dose fractionation, for instance by giving a different number of fractions, changing the fraction size or radiation dose rate, we can then investigate the therapeutic effects in term of *isoeffect plot* (Figure2D). Experimentally this is done by performing multiple studies at different doses for each chosen schedule and calculating a dose-response curve. We then select some particular level of effect (T in Figure2C) and read off the total radiation dose that gives this effect. For effects on normal tissues the isoeffect will often be some upper limit of tolerance of the tissue, perhaps expressed as a probability of tissue failure. The isoeffect plot show how the total radiation dose for the chosen level of effect varies with dose schedule. The dashed line in Figure2D illustrates how therapeutic conclusion may be drawn from isoeffect curves.

3.4 Effect of Radiotherapy on DNA:

The irradiation of a biological system initiates a series of processes that can be classified in term of time scale over which they act. The physical, chemical and biological phases of this process have been described.

An electron with energy of 1 MeV has a range in soft tissue of a few millimeters [1.2]. In the early part of its track the particle moves very quickly and its rate of energy deposited is low; the result is a relative straight track in which the ionizations may be separated by distance of around 0.1mm on average. We describe this as radiation with a low linear energy transfer (LET). As the electron slows down, it interacts more strongly with the orbital electrons in the medium. Its rate of energy loss increase, the track becomes more tortuous due to the stronger collision, and the ionization density increase. Figure3A shows a computer simulation of the tracks of 1 KeV electrons, representing a very small part of the tracks of 1 MeV electrons. The important feature is

the tendency towards clustering of the ionization events at the end of the track, each cluster having the size of a few nanometers. Within each electron track there is opportunity of interaction between the products of separate ionization events and it may be, particularly at low dose rate or following acute radiation doses up to few Gy, that the main biological effects of radiation (i.e. cell killing and mutation) are predominantly due to damage that is produced by these 'hot spots'. Within perhaps 10^{-10} seconds of exposure to either photon or particles beam, the irradiated volume will contain atoms that have been ionized and a corresponding number of free electrons, all produced by cascade of atomic reaction just described and with a rather non-uniform spatial distribution. The number of ionization produced at therapeutic dose levels is very large – approximately 10^5 ionizations per cell per Gy – but the vast majority of these produce no toxic damage. The biological effect is influenced by three factors: free radical scavenging processes, the number of ionizations that are closed to DNA to damage it, and the cellular repair process.

3.4.1 Indirect DNA damage:

Since biological systems consist largely of water, the bulk of the ionization produced by irradiation occurs in water molecules. Negatively charged free electron that are produced by ionization will rapidly become associated with polar water molecules, greatly reducing their mobility. The configuration of an electron surrounded by water molecules (a 'hydrated electron e^-_{aq}) has a degree of stability and lifetime under physiological condition of few microseconds. The water molecule that has lost an electron is a highly reactive positive charged ion. It quickly breaks down to produce a hydrogen ion (H^+) and an (uncharged) OH radical. OH is a molecule that normally doesn't exit in water, indeed the stable configuration is H_2O. The uncharged OH radical has an unpaired electron ('unattached valence') that makes it highly reactive. We designate it as a free radical thus: $OH^\bullet$. Free radicals are simply fragment of broken molecules. $OH^\bullet$ is different from OH^+ which is positive charged ion: the OH radical has equal number of protons and orbital electrons but because of unpaired electron is chemical reactive (some ions may also be radical, for example a water molecule that has lost an electron is actually H_20^+, a radical cation). Similarly, H+ is a bare proton, positively charged, whilst $H^\bullet$ is a proton plus an electron (neutral charge) but again highly reactive because the stable form of hydrogen is H_2.

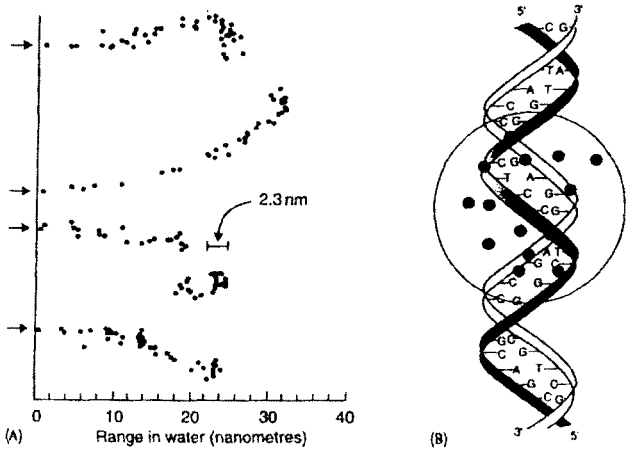


Figure3: (A) Computer-simulated tracks of 1 KeV electrons. Note the scale in relation to the 2.3 nm diameter of DNA double helix (adapted from Chapman and Gillespie, 1981). (B) Illustrating the concept of a local multiply damaged site produced by a cluster of ionizations impinging on DNA

Around 10^{-10} seconds after irradiation there will be three principal radiolysis products of water: e^-_{aq}, $OH^\bullet$ and $H^\bullet$. These highly reactive species will go on to take part in further reactions. An important one is:

$$OH^\bullet + OH^\bullet \rightarrow H_2O_2$$

the production of hydrogen peroxide. Oxygen, if present, plays an important part in the free-radical reactions following the irradiation. Molecular oxygen has a high affinity for free-radical ($R^\bullet$):

$$R^\bullet + O_2 \rightarrow RO^\bullet_2$$

giving rise to further reactive products and acting to fix the free-radical damage. The oxygen effect in radiation cell killing has often been explained in term of this type of process.

In biological system the free radicals produced in water may react with essential macromolecules. A vast range of reaction takes place, most of which are unimportant for the survival and functioning of the cell. The most important reactions are those with DNA, because of the uniqueness of many parts of this molecule. Damage of DNA by free radicals produced in water is called the *indirect* effect of radiation; ionization of atoms that are part of the DNA molecule is the *direct* effect (A Boriano, C Peroni; 2005).

3.4.2 Direct damage of DNA

Early experiments showed that irradiation leads to a loss of viscosity in DNA solutions. Subsequently this has been shown to result from DNA strand breaks. There are two categories of DNA strand breaks: single-strand (SSB) and double-strand (DSB). The detection of these depends on a study of the size distribution of fragments of DNA after extraction from irradiated cells. As

shown in Figure4, there is a variety of other types of DNA lesion that may have a role in cellular responses to radiation or chemical damage.

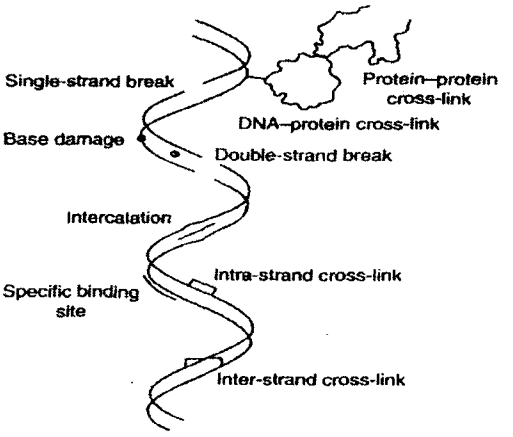


Figure4 Types of damge to DNA produced by radiation and chemical agents.

There are many sources of evidence to suggest that DNA damage is the critical event in radiation cell killing and mutation, including the following:

Micro-irradiation studies show that to kill cells by irradiation of only the cytoplasm requires far higher radiation dose than irradiation of the nucleus.

Isotopes with short-range emission (such as ^{3}H, ^{125}I) when incorporated into cellular DNA efficiently produce radiation cell killing and DNA damage.

The incidence of chromosomal aberrations following irradiation is closely linked to cell killing.

The number of lesions induced in DNA by radiation is far greater than those that eventually lead to cell killing. A dose of radiation that induces on average one lethal event per cell will kill 63% and leave 37% still viable (this result from Poisson statistic) and we call this the D_0 dose. D_0 values for oxic mammalian cells are usually in region of 1-2 Gy. The numbers of DNA lesions per cell that are detected immediately after such a dose have been estimated to be approximately:

Events per D_0	
Base damage	>1000
Single-strand breaks	~1000
Double-strand breaks	~40

In addition, cross-links between DNA strands and between DNA and nuclear proteins are formed (Figure4). Irradiation at clinical used doses thus induces a vast amount of DNA damage, most of which is successfully repaired by the cell. In a variety of experimental situations it has been found that the incidence of cell killing fails to correlate with the number of SSB induced, but relates better to the incidence of DSB. Significantly, a dose of hydrogen peroxide that induces many DDB produces little cell killing and few DSB unless the number of SSD is so large that they are close enough to form DSB. On this basis it is generally believed that DSB are the critical lesions

for radiation cell killing in most cell types, although experimental evidences indicate that only some DSB are important(A Boriano, C Peroni; 2005).

Modifier	Cell kill	DSB	SSB	Base	DNA-protein cross-link
High-let rad	↑	↑	↓	↓	-
Hypoxia	↓	↓	↓	0	↑
Thiols	↓	↓	↓	0	↓
Hypertermia	↑	↑	0	0	0
Hydorgen per.	0	0	↑	↑	-

↑, Increased; ↓, Decreased; 0 little or no effect; -, not know.

See Frankenburg Schwager (1989) for further information

Table 1 Double-strand DNA breaks correlate best with cell killng

The DNA damage response involves initial sensing of DSB, followed by a highly regulated signal transduction cascade that changes effector protein functions. Effector proteins regulate such things as changes in cellcycle progression and chromatin modification around sites of DNA damage. They also include recruitment of proteins needed for DNA repair, and also regulate, through either apoptosis or mitotic death, the subsequent fate of the cell or its progeny. The response to different types of DNA damage involves proteins with overlapping functions and other proteins with functions that are specific to a particular lesion or process. Abnormalities in the response have consequences for cellular radiation sensitivity as measured by clonal death and for spontaneous and radiation-induced genomic instability.

Although ionising radiation causes a plethora of lesions in DNA, there is evidence that the DNA DSB is the most important in terms of tumour and normal tissue radiosensitivity (A Tutt and J. Yarnoldy; 2005).

As few as one unrepaired DSB can stimulate apoptosis, and therefore sensing mechanisms are obviously sensitive and capable of stimulating a rapid cellular response. Evidence suggests that sensing mechanisms may distinguish between DNA lesions that can be rapidly repaired by DNA repair proteins and those that require a wider response, including cell-cycle checkpoint activation . Although the initial sensing molecules are largely unknown, the data suggest a general model whereby DNA end-binding proteins recruit members of a PI3-kinase-like kinase (PIKK) family to the damage site. These phosphorylate chromatin (histones) around the break site and downstream signal transduction proteins. These second-phase signal transducers recruit specific repair enzymes to the site of damage, and effect wider changes in cell-cycle progression and cell fate (A Tutt and J. Yarnoldy; 2005).

The repair of DSB by a process called non-homologous end joining (NHEJ) is fairly well understood. The free ends of DSB are initially bound and protected by heterodimers of two proteins named Ku 70 and Ku 86. These recruit and activate the catalytic subunit of a PIKK enzyme called DNA protein kinase (DNA-PKcs), which in combination with Ku recruits additional factors required for NHEJ. There is currently little evidence to suggest that DNA-PKcs or Ku function is abnormal in breast cancer. Although mutations in DNA-PK or Ku, through their roles in VDJ recombination in B cells, are responsible for the radiosensitive severe combined immune deficiency syndrome, mutations are not a recognised cause of normal tissue radiation toxicity in patients (A Tutt and J. Yarnoldy; 2005).

The end of this highly regulated signal transduction cascade involves proteins that are actually responsible for repair. Different DNA lesions are repaired by differing groups of effector proteins, with some shared between several processes. For DSB, there are thought to be three distinct repair pathways: (1) NHEJ, (2) Rad51-dependent homologous recombination, and (3) homology-directed singlestrand annealing. Each has different consequences for cellular survival and genome stability. The DNA DSB repair mechanisms are thought to be differentially regulated through the cell cycle, with NHEJ being active during G0/1 and early S phase. Rad51-dependent homologous recombination, requiring the presence of a sister chromatid template, occurs in the S and G2 phases. There is little knowledge of the cell-cycle dependence of the highly mutagenic homology-directed single-strand annealing mechanism (A Tutt and J. Yarnoldy; 2005).

3.5 Factors affecting radiotherapy:

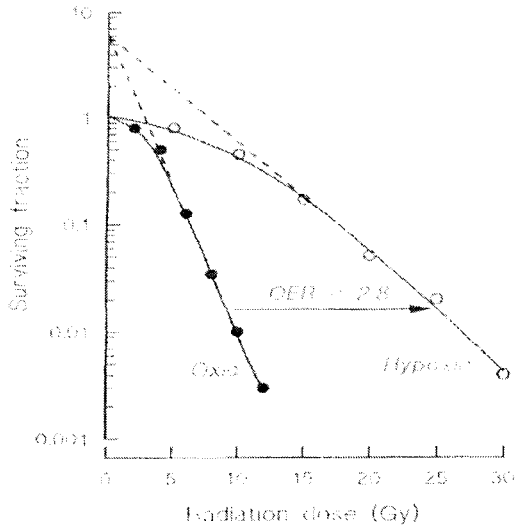
There are several factors that affecting in radiotherapy, as Oxygen, cell cycle, type of radiation (LET), Age of patient, depth of tuomor and fractionation. Here we discuss some of this factors and thir significant relationship.

3.5.1 The oxygen effect:

The response of cells to ionizing radiation is strongly dependent upon oxygen. The cell surviving fraction is shown as a function of radiation dose administrated either under normal aerated condition or under hypoxia, generally achieved by flowing nitrogen gas over the surface of the cells suspensions for a period of 30 minutes or more. The enhancement of radiation damage by oxygen is dose-modifying, i.e. the radiation dose that gives a particular level of survival is reduced by the same factor at levels of survival. This allows us to calculate an oxygen enhancement ratio (OER):

$$\text{OER} = \frac{\text{dose in } N_2 \text{ for surviving fraction, } S/S_0}{\text{dose in } O_2 \text{ for surviving fraction, } S/S_0}$$

for the same level of biological effect. For most cells the OER for x-rays is around 3.0. However, some studies suggest that at radiation dose of 3 Gy or less the OER is actually reduced. This is an important finding because this is the dose range for clinical fractionation treatments .



Survival curves for culturaed mammalian cells exposed to x-rays under oxic or hypoxic conditions, illustrated the radiation dose-modifying effect of oxygen. Note that the broken line extrapolate back to the same point on the survival axis (n=5.5).

It has been demonstrated from rapid-mix studies that the oxygen effect only occurs if oxygen is present either during irradiation or within a few millisecond thereafter (Howard-Flanders and Moore, 1958). The dependence of the degree of sensitization on oxygen tension is shown in Figure.5. By definition, the OER under anoxic condition is 1.0. As the oxygen level increases, there is a steep increase in radiosensitivity (and thus in OER). The greatest change occurs from 0 to about 20 mmHg; further increase in oxygen concentration, up to that seen in air (155 mmHg) or even to 100% oxygen (760 mmHg), produces a small though definite increase in radiosensitivity. Also shown in Figure.5 is the oxygen partial pressure range typically found in arterial and venous blood. Thus, from a radiobiological standpoint most normal tissues can be considered to be well oxygenated, although it is now recognized that moderate hypoxia is a feature of some normal tissue such as cartilage and skin.

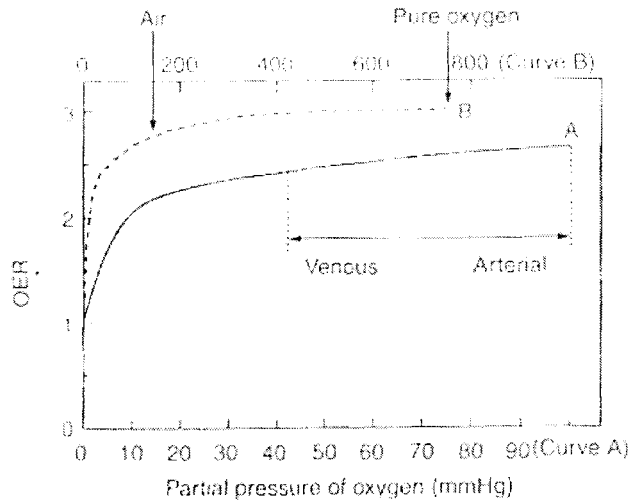


Figure.5 Variation of oxygen enhancement ratio (OER) with oxygen tension. The horiziontal arrows indicate the range of physiological blood oxygen tensions on the lower scale(A Boriano, C Peroni; 2005).

The mechanism responsible for the enhancement of radiation damage by oxygen is generally referred to as the oxygen-fixation hypothesis and is illustrated in Figure.6. When radiation is absorbed in a biological material free radicals are produced. These are highly reactive molecules and can thus break chemical bonds, produce chemical changes, and initiate the chain of events that results in biological damage. They can be produced either directly in target molecule (usually DNA) or indirectly in other cellular molecules and defuse far enough to reach and damage critical targets. Most of the indirect effects occur by free radical produced in water, since this makes up to 70-80% of mammalian cells. It is the fate of the free radicals ultimately produced in critical target, designated in $R^{\bullet}$ in Figure.6, that is important. If oxygen is present, then it can react with $R^{\bullet}$ to produced $RO_2^{\bullet}$ which then undergoes further reaction ultimately to yield ROOH in target molecule. Thus we have a change in the chemical composition of the target and the damage is chemically fixed. Subsequently this damage can be processed enzimatically and perhaps repaired. In the absence of oxygen, or in the presence of reduced species, $R^{\bullet}$ can react with H^{+}, thus restoring its original form.

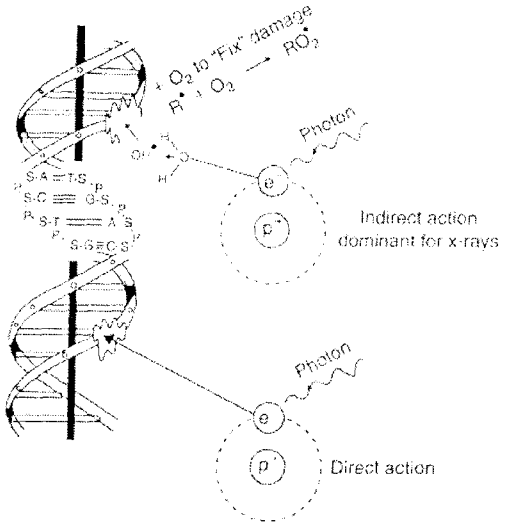


Figure.6 The oxygen fixation hypothesis. Free radical produced in DNA either by direct or indirect action of radiation can be rapired under hypoxia but fixed in the presence of oxygen.

The degree of hypoxia in tumors has been shown to be a negative prognostic indicator in a variety of cancers, particularly when the modality is radiotherapy, but also for other modalities. For radiotherapy, one obvious contributing factor is that hypoxic cells are up to threefold more resistant to ionizing radiation than cells irradiated under well-oxygenated conditions. This phenomenon has stimulated considerable effort for several decades to find ways to sensitize hypoxic cells specifically to ionizing radiation or to develop drugs specifically toxic to hypoxic cells. These have led to improvements in responses in some clinical trials, confirming the importance of hypoxic cells in determining treatment outcome, and in particular, the importance of radioresistance. The present hypoxic radiosensitizers and hypoxic cytotoxins have associated toxicity and are not optimal in terms of efficacy. The search for better methods to attack the hypoxia problem is therefore an important ongoing endeavor. To design better interventions to overcome hypoxic radioresistance, an understanding of the mechanism of resistance is important. Radiochemical mechanisms determining resistance have been known for many years to play an important role. In brief and simplistically, under normoxic conditions, oxygen is capable of reacting with electrons ejected from macromolecules by the irradiating photons (ionizations), preventing return and recombination on the ionized macromolecule—essentially fixing the damage radiochemically. Hydrogen donation by thiols, particularly glutathione, can "repair" lesions, but such thiols compete inefficiently with the counteracting effects of oxygen. The lack of oxygen in hypoxic cells results in a greater probability of radiochemical restitution or "repair," and thus less induced damage. The time scale of this process is in milliseconds. More recent evidence, however, has suggested that biochemical, as well as radiochemical, mechanisms may be affecting the radioresistance of hypoxic cells. In addition to the more numerous DNA base damages, DNA single-strand breaks and DNA double-strand breaks, ionizing radiation also

induces DNA–protein cross-links and DNA–interstrand cross-links. DNA–protein cross-links are one of the few lesions that have been shown to be formed in greater numbers under hypoxic than under normoxic irradiation conditions. Murray and colleagues showed a correlation between hypoxic radioresistance and the sensitivity of the cells to DNA interstrand cross-linking agents in a series of hamster mutant cell lines. The implication is that the ability to repair cross-links is important for radioresistance under hypoxia but less so for normoxic irradiation, because fewer crosslinks are formed. The repair of such cross-links takes hours rather than fractions of 1 second. Many aspects of repair pathways involved in removal of interstrand cross-links are now known. These involve some, but not all, proteins central to nucleotide excision repair (NER), namely ERCC1 and XPF. These proteins form a dimer, stimulating activity capable of incising DNA during NER, 5= of a lesion such as a bulky adduct or thymine dimer. Cells mutated or lacking either of these two genes are highly sensitive to interstrand crosslinking agents. Murray and colleagues exclusively studied mutants on the NER pathway. In addition, however, homologous recombination (HR) appears to play a central role in cross-link repair, because cells lacking one of several genes central to HR also exhibit considerably enhanced sensitivity to cross-linking agents. So Homologous recombination plays a greater role in determining hypoxic radiosensitivity than normoxic radiosensitivity. This is not the case for nonhomologous end-joining. The effect is probably mediated by repair of interstrand cross-links, although direct evidence of increased cross-link yields under hypoxia in mammalian cells is lacking. Effects of HR on DNA-protein cross-links, for which good evidence of increased yields under hypoxia exists, remains a possibility. These imply that targeting HR to increase radiosensitivity would provide partial tumor selectivity, because of proliferation and hypoxia dependencies (Debbie Sprong, *et al*, 2006).

Most in vitro experiments have been performed measuring clonogenic survival after a range of relatively large single doses, e.g. 5–15 Gy in oxygen and 15–45 Gy in hypoxia. Such experiments have yielded *OER* values of 2.5–3. It is often simplistically assumed that the *OER* is truly a dose modifying factor that applies over the entire dose range that can be used in assaying cell survival. Therefore, the *OER* values obtained with aerobic radiation doses of 5–15 Gy have often been applied to the much lower 2 Gy doses used in each daily fraction in the clinic, e.g. when modelling the response expected of tumours and normal tissues in vivo. The actual value of the *OER* at low doses is critical in determining the impact of hypoxia as a potential cause of resistance in tumours exposed to repeated small doses of radiation in radiotherapy (A. Das and J. Denekamp, 1997).

3.5.2 Particle beams in radiotherapy

Figure7 shows examples of microdosimetric calculation of ionization tracks from γ-rays or α-particles passing through a cell nucleus (Goodhead, 1988). At the scale of cell nucleus, the γ-rays deposit much of their energy as single isolated ionizations or excitations and much of resulting of DNA damage is efficiently repaired by enzymes within the nucleus. About 1000 of these sparse tracks are produced per Gy of absorbed dose. The α-particles produce fewer tracks but the intense ionization within each tracks leads to more severe damage where the track intersects vital structure such as DNA. The resulting DNA damage may involve several adjacent base pairs and will be much more difficult or even impossible to repair; this is probably the reason why these radiations produce steeper cell survival curves and allow less cellular recovery than x-rays. At the low doses that are encountered in environmental exposure, only some cells will be traversed by a particle and many cells will be unexposed [1.5].

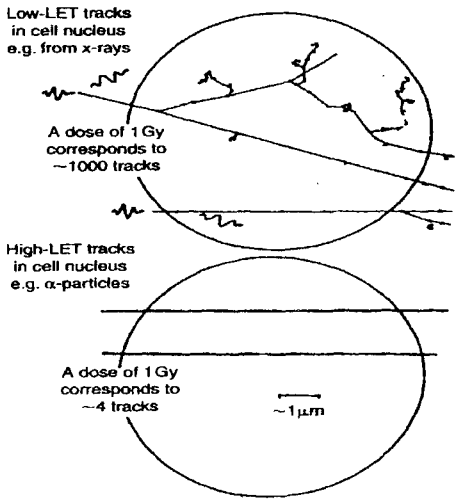


Figure7 The structure of particle tracks for low-LET radiation (above) and α-particle (below). The cricles indicate the typical syze of mammalian cell nuclei. Note the tortuos tracks of low-energy secondary electrons(A Boriano, C Peroni; 2005).

Linear energy transfer (LET) is the physical quantity used to describe the density of ionization in particle tracks. LET is the average energy given up by a charged particle traversing a unitary distance, normally expressed in $KeV\mu m^{-1}$. In Figure7 the γ-rays have a LET about 0.3 $KeV\mu m^{-1}$ and are described as low-LET radiation. The α-particles have a LET of about 100 $KeV\mu m^{-1}$ and are an example of high-LET radiation.

Also neutrons are describes as high-LET radiation, even if they are uncharged. In fact they do not interact with the orbital electrons in the tissue through which they pass and they do not directly produce ionization. They do, however, interact with atomic nuclei from which they eject slow, densely ionization protons. It is this secondary production of knock-on protons that confers high LET(A Boriano, C Peroni; 2005).

The rationale for introducing ion beams in cancer therapy is two-fold: first a high physical selectivity can be achieved with ions, comparable to (or even better than) proton beams or modern photon therapy techniques such as conformal therapy, IMRT, and modern brachytherapy. Second, there is the potential advantage of high-LET radiations in reducing the photon-resistance of certain tumours. High-energy proton beams are low-LET radiation, as are photon- and electron beams. Fast neutrons, neon- and carbon ions are the high-LET radiations that have been used in radiation therapy. Radiation quality remains rather constant as a function of depth in neutron beams, while the LET increases significantly with depth in the ion beams as the energy of the particles decreases. Fast neutrons were often applied under suboptimal technical conditions, and this aspect must be taken into account in retrospective evaluation of the clinical neutron data. In contrast, with ions, a high level of physical selectivity can be achieved because of the Bragg peak (A Wambersie *et al*; 2003).

The rationale for introducing ion beams in cancer therapy is the high level of physical selectivity that can be achieved with ions, equal or even better than with proton beams or modern photon techniques, as well as the potential advantage of high-LET radiations for some tumour types and sites. The radiobiological arguments for high-LET radiation in cancer therapy are reviewed: reduction of OER in the case of hypoxic and poofly-reoxygenating tumours, and the lesser importance of repair phenomena which are a problem in controlling repair-proficient photon-resistant tumours. Fast neutrons were the first type of high-LET radiation used clinically, and were often applied under suboptimal technical conditions. Nevertheless, useful clinical information was derived from the neutron experience. A greater benefit from neutrons than from conventional radiotherapy was found for several tumour sites (A Wambersie *et al*; 2003).

Radiation quality is defined by the nature, charge and energy spectrum of the particles and can be characterized by the Linear Energy Transfer (LET) and/or the microdosimetric spectra at the point of interest under the actual irradiation conditions. The differences in energy distribution between high- and low-LET radiations, at the level of the subcellular structures, are reviewed elsewhere. Comparison of the microdosimetric spectra for equal dose indicates that the energy deposited in traversed subcellular volumes of around 1 or 2 pm in diameter is, as an average, about 100 times higher for example for fast neutrons than for gamma rays (A Wambersie *et al*; 2003).

These large differences in energy distribution, at the microscopic level and at equal absorbed dose, result in different biological effects dependant on the radiation quality. Some can be predicted: a higher RBE (ranging between 3 and >>5 in the conditions of fast neutron therapy), different shapes of the dose-effect relationships for tumours and normal tissues and hence the possibility to obtain a differential effect and a therapeutic benefit (A Wambersie *et al*; 2003).

As LET increases, radiation produces more cell killing per Gy. Figure.8 shows the survival of human T1G cells plotted against dose for high different radiations, with LET varying from 2

keVμm^{-1} (250 kVp x-rays) to 165 keVμm^{-1} (2.5 MeV α particles). As LET increases, the survival curves became steeper; they also become straighter with less shoulder, which indicates either a higher ratio of lethal to potentially lethal lesion (in lesion-interaction models) or that high-LET radiation damage is less likely to be repaired correctly. For particles of identical atomic composition, LET generally increases with decreasing particles energy. However, notice that 2.5 MeV α particles are less efficient compared with 4 MeV α particles even though they have a higher LET; this is due to the phenomenon of overkill indicated in Figure9.

The relative biological effectiveness (RBE) of a radiation under test (e. g. a high LET radiation) is defined as:

$$\text{RBE} = \frac{\text{dose of reference radiation}}{\text{dose of test radiation}}$$

to give the same biological effect.

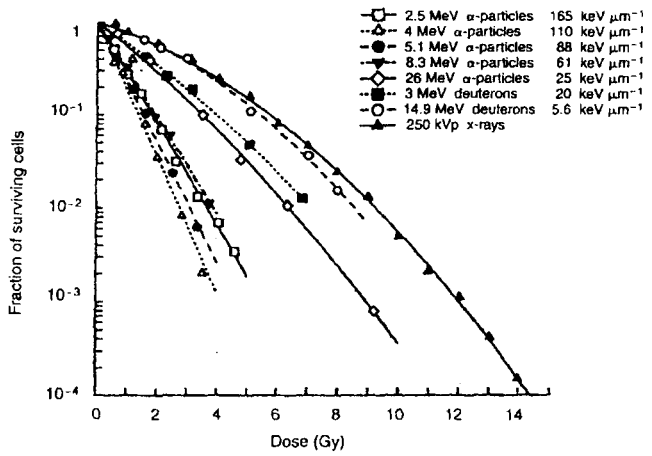


Figure.8 Survival of human kidney cells exposed in vitro to radiations of different LET. (A Boriano, C Peroni; 2005).

The reference low-LET radiation is usually 250 kVp x-rays. Figure9 shows RBE values for T1g cells featured in Figure.8. Curves have been calculated at cell survival levels of 0.8, 0.1 and 0.01, illustrating the fact that RBE is not constant but depends on the level of biological damage and hence on the dose level. RBE rises to a maximum at a LET of about 100keVμm^{-1}, then falls for higher values of LET due to overkill. For cells to be killed, energy must be deposited in a number of critical sites in the cell.

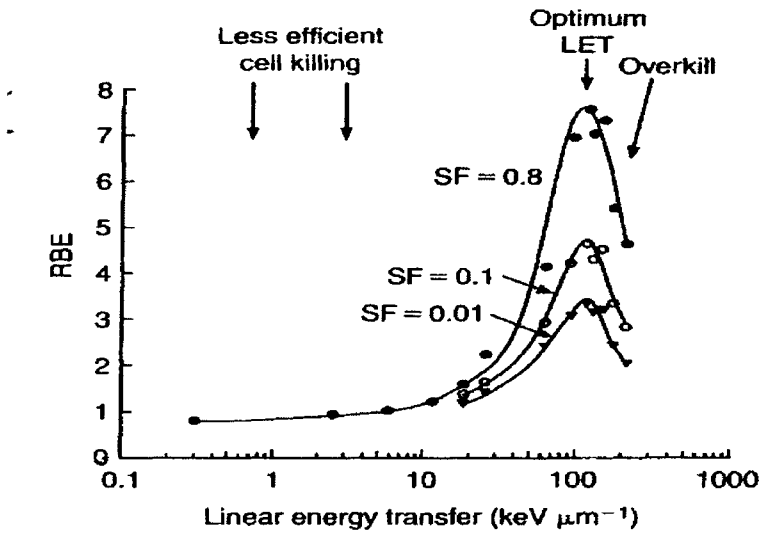


Figure9 Dependece of RBE on LET and the phenomenon of overkill by very high-let radations.

Sparsely ionizing low-LET radiation is inefficient because more than one particle may have to pass through the cell to kill it. Densely ionizing very high-LET radiation is also inefficient because it deposits more energy than necessary in critical sites. These cells are overkilled and per Gy there is then less likelihood that other cells will be killed, leading to a reduced biological effect. Radiation of optimal LET deposits just enough energy per cell to inactivate the critical targets. This optimum LET is usually around 100mm-1 but it does vary between different cell types and depends on the spectrum of LET values in the radiation beam as well as the mean LET. As LET increases, the oxygen enhancement ratio decreases. The measurements shown as an example in Figure.10 were also made with T1g cells of human origin. The sharp reduction of OER occurs over the same range of LET as the sharp increase in RBE (Figure9).

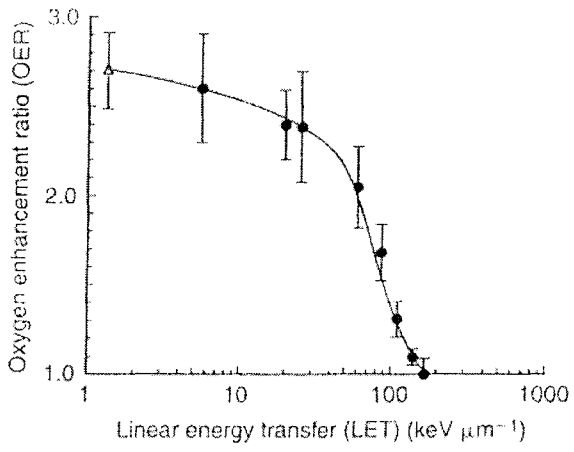


Figure.10: The oxygen enhancement ratio (OER) decreases with increasing LET. Closed circles refer to monoenergetic α-paritcles and deuterons; the open triangle to 250 kVp x-rays.

3.5.3 Oxygen Enhancement Ratio (OER) and LET:

Historically, the oxygen effect was the rationale for introducing fast neutrons in radiotherapy:

(i) hypoxic clonogenic cells are present in malignant tumours, generally at the level of a few percent; they result from the fast and anarchic proliferation of the cancer cells. There is chronic hypoxia in cells at the periphery of tumour cords around the microvasculature, as well as transient hypoxia in whole cords caused by the transitory closure of these blood vessels.

(ii) hypoxic cells are =2-3 times more radioresistant than well oxygenated cells (OER=2-3) with low-LET radiation. The presence of a small percentage of hypoxic cells (1% or even 0.1%) can make the tumour radioresistant to high single doses. If the reoxygenation phenomenon during fractionated treatment is inefficient, the continuing presence of hypoxic cells may make the tumour resistant to cure using photons.

(iii) the OER decreases when LET increases. It decreases down to about 1.6 for fast neutrons, and is close to unity for alpha particles (OER=1.3 and 1.0 for alpha particles of 4 and 2.5 MeV, i.e. for 110 and 160 keV/#m, respectively).

When carbon ions are spread out to irradiate a tumour volume, the high-LET component is diluted and the resultant OER is about 1.5, a value similar to that for neutrons. Hence the potential for better irradiation of hypoxic compared to oxic cells is not improved with ions compared to neutrons, but this feature is in addition to the much improved dose distribution with ions and the high biological dose (RBE>I) delivered to the planning target volume (PTV) in the spread-out Bragg peak (SOBP). A reduction in OER should be in principle nearly always an advantage when treating poorly reoxygenating turnouts, because most normal tissues are either well oxygenated or are homogeneously slightly hypoxic. A notable example of normal tissue hypoxia is laryngeal cartilage, sensitized by hyperbaric oxygen treatment. Attempts to deal with the potential therapeutic problem of radioresistant hypoxic tumour cells have had various degrees of success e.g. hyperbaric oxygen increased tumour control rates by on average 10 % , radiosensitising chemicals such as nimorazole by 11-16 % [45land other bioreductive agents cytotoxic for hypoxic cells have shown promise. However, the use of these agents is still not widespread. However, it is recognized that there is variability in hypoxia between tumours, even between turnours of the same type in the same site. The fraction of tumours that are the most hypoxic will benefit most from these procedures, and benefit more than the average amount for all tumours (AWambersie *et al*; 2003).

Cells in stationary phase and in S phase are significantly more radioresistant than G2/M cells. Increasing LET reduces the differences in radiosensitivity related to the position of the cells in the mitotic cycle. Hence variability in cell radiosensitivity in the mitotic cycle is reduced, but any benefit of this reduction for high-LET therapy remains unclear (A Wambersie *et al*; 2003).

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