General Introduction
Cancer is a major health problem, with 14.1 million new cases and 8.2 million deaths due to cancer worldwide in 2012 alone (1). The number of new cases per year is estimated to rise to 22.2 million by the year 2030 (2). Therefore, it is necessary to continually advance and develop cancer treatments, to better cope with increasing numbers of patients and to attain a better level of patient care post-treatment. The three oldest and most common cancer treatment modalities are surgery (3), chemotherapy (4) and radiotherapy (5), with more modern treatments, such as immunotherapy (6-8), being developed.

Radiotherapy is used (either alone or in combination with other forms of treatment) in approximately 50% of all cancer patients (9, 10). The objective of radiotherapy as a cancer treatment is to administer the greatest dose possible to the tumor while limiting the dose to the surrounding normal tissue (5). This balance between the benefit of radiotherapy and the risk to normal tissue is known as the therapeutic window. However, there are many factors which limit the ability to optimally deliver radiation doses. Nowadays, developments are continuously taking place to further enhance the effects of radiotherapy. As techniques are developed to optimize the efficacy of radiotherapy, it is also necessary to develop new experimental models to better understand the mechanisms that determine the limitations of radiotherapy and how new developments impact these mechanisms.

Limitations of radiotherapy
The therapeutic outcome of radiotherapy is influenced by many factors. These factors include variables such as availability of oxygen, stage of tumor differentiation, presence of cancer stem cells, rates of cellular proliferation and initiation/capacity of DNA damage responses. While all these factors potentially contribute to the effects of radiotherapy on tumor cells, they may also play a role in the response of normal tissue to unavoidable co-irradiation and consequently limit the dose which can be delivered to the tumor.

Mechanisms of radioresistance
To enhance tumor elimination while minimizing the adverse side effects caused to normal tissue, removal of cancer stem cells (CSCs) is necessary, as these are the cells which are resistant to many anticancer therapies, including radiation therapy. Moreover, CSCs are capable of regenerating a tumor following treatment. Features of the tumor microenvironment, such as hypoxia (10), and intracellular signal transduction pathways (11) are all factors that may contribute to tumor radioresistance.
Furthermore, CSCs themselves are often found to be intrinsically more radioresistant than non-stem cells of the tumor, often due to the same factors listed above (11-13).

Regions of varying oxygen levels are often found within a tumor, and cells within hypoxic regions are frequently more resistant to anti-cancer therapy than cells in regions with higher oxygen levels. One mechanism that is suggested to contribute to the increased radioresistance in hypoxic regions compared to normoxic conditions is the ‘oxygen fixation hypothesis’, first proposed by Alexander and Charlesby in 1954 (14). Under normoxic conditions DNA lesions generated by ionizing radiation are ‘fixed’ by oxygen to form more ‘unrepairable’ DNA damage, which induce cell death. However, under hypoxic conditions DNA radicals are not fixed by oxygen (or to a lesser degree), leading to less permanent DNA damage, and thus the induction levels of cell death are reduced (10).

Furthermore, hypoxia stabilizes the transcription factor hypoxia inducible factor 1α (HIF-1α). Upon stabilization, HIF-1α translocates to the nucleus where it forms a dimer with HIF-1β. HIF-1 binds to DNA and induces its downstream targets which promote cell survival (15), and thus further counteracts the toxic effects of radiation treatment. Therefore, the two primary causes of radioresistance in hypoxic regions are due to the influence of hypoxia on DNA damage and cell survival mechanisms. Furthermore, hypoxia and HIF-1α may also contribute towards the inherent resistance of CSCs (16, 17). Hypoxic regulation of signalling downstream of mammalian target of rapamycin (mTOR) contributes to autophagy and thus induces stem-like properties, including treatment resistance (18). An increase in autophagy due to HIF-1α activation has been shown to induce non-CSCs to transform CSCs (or at least CSC-like cells) (19, 20).

Changes in many intracellular signalling pathways have also been shown to promote radioresistance in tumor cells. For example, insulin-like growth factor 1 (IGF-1) overexpression is associated with radioresistance and tumor recurrence in breast cancer (21), while epidermal growth factor (EGF) and its receptor (EGFR) have to been shown to be associated with radioresistance in prostate carcinoma cell lines (22), head and neck squamous cell carcinomas (23, 24) and glioma cells (25, 26), amongst others. Another cytokine, transforming growth factor beta (TGF-β), has also been shown to enhance radioresistance of esophageal cell lines (27). Furthermore, mutations in the p53 tumor suppressor pathway have long been known to induce radioresistance (28). These pathways play various physiological roles in inflammation, promoting cell survival, cell division, to promoting angiogenesis,
and thus may lead to resistance or repopulation after treatment. Targeting these pathways may offer a promising means to enhance the radiosensitivity of a tumor.

Further understanding of the mechanisms behind radioresistance may reveal novel cues for enhancing tumor response to (chemo-)radiotherapy. New models for studying radioresistance, especially CSC radioresistance, may allow for the identification of patients who may respond to radiotherapy prior to undergoing treatment.

**Normal tissue side effects**

Normal tissue side effects can be divided into early and late side effects based on when they occur and the severity of the consequences. Early (or acute) side effects occur during, immediately after or soon after (within weeks) radiotherapy treatment (29). These side effects occur due to DNA damage induced cell death of highly proliferative cells with a fast turnover, resulting in inflammation. Early side effects may be (partly) reversible and include pneumonitis (inflammation of the alveolar walls in the lungs) (30, 31), erythema (32), memory loss, fatigue (33), diarrhoea (29) and oral mucositis (34).

Late normal tissue side effects occur several months, to even years, after radiation treatment (29). Late side effects are generally chronic (and often become worse over time) and are therefore often dose limiting for radiotherapy (29). It has been shown by numerous *in vivo* animal studies and clinical studies that normal tissue side effects show a dose-to-volume relationship. For example, following irradiation for head and neck cancers, xerostomia is the result of late and acute side effects of the submandibular and parotid salivary glands in particular and is well-known to impact greatly on the quality of life of patients (35-37).

Another well-known late side effect with a dose-to-volume relationship is ischemic heart disease (38, 39). Darby et al. showed the risk of ischemic heart disease increases proportionally with the mean dose to the heart during radiation treatment for breast cancer (40). Other chronic side effects that may impact on the quality of life following radiation treatment include radiation induced fibrosis (41). Radiation induced fibrosis can occur in the heart (42), lung (30, 43), liver (44), or any tissue which lies in the field of therapy.

While damage/destruction of tissue is again the cause of many late side effects, contrary to early
side effects, the damage is frequently irreversible and, as stated, has dramatic effects on the quality of life of patients following treatment. Normal tissue stem cells are essential for the homeostasis of tissue which is affected by radiation therapy (45). Therefore, further insights into the mechanisms and consequences of normal tissue side effects, particularly with a focus on normal tissue stem cells, will allow for better patient care in the future. Although in vivo studies offer the most complete insights into the mechanisms behind normal tissue damage, the development of further in vitro models for studying radiotherapy will provide an invaluable source of evidence into mechanisms of normal tissue side effects that may not be easily discovered in vivo.

**Low dose hyper-radiosensitivity and increased radioresistance**

Optimal treatment planning for conventional radiotherapy (using X-rays) treatments targets the maximum irradiation dose (usually 1.8-2 Gy/fraction up to doses of 72 Gy or even more) to the tumor. This results in potentially large volumes of normal tissue surrounding the tumor receiving low doses, often below 1 Gy/fraction (as depicted in figure 1), which can potentially elicit a low dose response.
Mathematical models, such as the linear quadratic model, for the prediction of cell survival following irradiation generally assume that survival (and thereby functional) responses will decrease continuously with increasing radiation doses (46-48). However, both in vitro and in vivo, there are many indications that responses deviate from these predictions (49). Deviation from the predicted response was first described by Marples and Joiner in Chinese hamster V79 cells in which they saw a steeper decrease in survival at low doses (below 0.6 Gy) than at fractionally higher doses (>0.6 Gy) (50). This phenomenon is frequently known as low dose hyper-radiosensitivity (LDHRS) and increased radioresistance (IRR) (49). Wouters et al. studied a panel of tumor cell lines and observed similar responses in the majority of these, showing that this was not a cell type specific phenomenon (51). Furthermore, Wouters et al. suggested that a dose-dependent difference in the handling of DNA damage induced by photons resulted in the subsequent resistance at higher doses (51).

Although the exact mechanism behind the increased survival at slightly higher doses is not known, many studies point towards the role of an induction of DNA damage responses at higher doses. Short et al. first identified a role of the cell cycle phase in affecting the presence/absence of LDHRS in T98G glioblastoma cells (52) and later identified this to be most prominent in G2 (53), with Krueger et al. suggesting this was due to evasion of the G2 checkpoint dependent of ATM (54). Despite a lack of a full understanding of the mechanism for LDHRS/IRR, this phenomenon may still be of significant importance for patient treatment planning.

Attempts have been made to harness the effects of LDHRS/IRR to enhance the efficacy of radiation of tumors, particularly tumors which are known to be radioreistant, such as glioblastoma. Beauchesne et al. performed a phase II trial of ultra-fractionation (3 daily fractions of 0.75 Gy each, 5 days a week for 6 weeks) to treat unresectable glioblastoma (55). This study showed an increased benefit and prolonged survival compared to radiotherapy fractions of 2 Gy per day for 6 weeks (55). While this study shows the potential importance of LDHRS/IRR in clinical tumor treatment planning, very little has been done to study the role of LDHRS/IRR in normal tissue.
If present, LDHRS/IRR in normal tissue may have a large impact on normal tissue side effects, as normal tissue surrounding a tumor may receive multiple low doses (often below 0.5 Gy). Hamilton et al. investigated erythema (a common early side effect of the skin) following doses of less than 2 Gy (32). Below doses of 1.5 Gy, the linear quadratic model for predicting radiation response, significantly deviated from the predicted erythema response (32), indicating the presence of LDHRS/IRR in normal tissue. These results show that LDHRS/IRR may indeed be present in normal tissue, however there is still much left to understand of LDHRS/IRR in normal tissue.

Furthermore, the impact of LDHRS/IRR has not been investigated in normal tissue stem cells. Normal tissue stem cells are the most important cells for the regeneration and function of tissues co-irradiated during radiotherapy treatment (45, 56, 57). In the future, the role of LDHRS/IRR in normal tissue stem cells should be evaluated, to enable the most effective normal tissue sparing. Appropriate in vitro stem cell models may allow for this.

**Stem cell models in radiotherapy**

As mentioned above, stem cells are the most important cells required for regeneration of tissues damaged by radiation treatment (depicted in figure 2). Due to this integral role of stem cells, stem cell therapy in patients to alleviate normal tissue side effects is not a new concept (58). Transplantation of bone marrow cells to replace the hematopoietic system following total body irradiation has already been used as part of the treatment of leukaemia since it was first described in mice by Vos et al. in 1956 (59) and subsequently in humans by Thomas et al. in 1957 (60). However, stem cell transplantation other than bone marrow for normal tissues affected by radiation is still a relatively new field, but there are highly promising preclinical data to show the strength and potential of stem cell therapy.
Figure 2: Tissue homeostasis. In normal healthy tissues, there is a balance between cell proliferation driven by stem cells and cell death to keep tissues under homeostatic conditions. Following damage, normally an increase in cell death is balanced by an increase in stem cell driven proliferation. However, if this damage is induced by irradiation stem cells may be depleted, shifting the balance towards cell death, leading to irradiation-induced side effects and reduced tissue functionality.
Recent *in vivo* mouse studies have shown that transplantation of human embryonic stem cells and human neural stem cells can alleviate radiation-induced cognitive impairment (61, 62), while the potential of mesenchymal stem cells to protect against radiation-induced late side effects of the lung in mouse models has also been revealed (63, 64). Furthermore, our group has shown the capacity of cultured salivary gland organoid cells, derived from both mouse and human, to restore the function of radiation-damaged salivary glands in mice (56, 57, 65, 66). These studies hold significant promise for the treatment of radiation-induced side effects.

While regenerative medicine is a field with great potential for the amelioration of late radiotherapy side effects, *in vitro* stem cell cultures have also opened new and exciting avenues in the fields of drug discovery and disease development (67-69). Organoids (*in vitro* 3D cell culture models derived from organ specific stem cells, which resemble mini-organs of their origin) offer the previously unprecedented opportunity to study organ specific drug interactions and furthermore hold great potential in the field of personalized medicine (depicted in figure 3) (70, 71).

![Figure 3: Organoids: a potential model to study radiation-induced side effects. Radiation-induced side effects observed at an organ and tissue scale are induced by damage in single cells, at a DNA level. Organoid models have been developed for many of the critical organs/tissues that are affected by radiation-induced side effects, and potentially offer a more physiologically relevant model to study radiation-induced side effects than adherent cell lines.](image-url)
Since identifying Lgr5 as a gastro-intestinal stem cell marker (72), Clevers and colleagues have developed many stem cell ‘mini-gut’ models, including intestinal (73) and colon (74). These models enabled Dekkers et al. to very elegantly show restoration of a functional CFTR gene (a cystic fibrosis transmembrane conductance regulator) in rectal tissue-derived organoids from cystic fibrosis patients (75). Treatment with Forskolin resulted in rapid swelling in healthy donor derived organoids, whereas swelling was reduced in organoids from patients with a mutation in CFTR (75). Furthermore, using this organoid model it was possible to restore a functional CFTR gene by chemical, temperature-based (75) and gene-editing methods (76), and to show differential drug responses between patient-derived organoids (77). These studies show the potential of using organoids models for diagnosis, gene-cell therapy, and drug discovery.

Many other groups are also successfully using organoid models for drug and toxicity studies in both healthy and cancer derived models. Using kidney organoids derived from induced pluripotent stem cells, Takasato et al. showed the known nephrotoxicity of cisplatin (78) and thus showed the potential using organoid models to identify nephrotoxicants in the future. Using patient-derived organoids for pancreatic adenocarcinoma, Huang et al. showed patient-specific sensitivities to an inhibitor of EZH2 (an epigenetic regulator), which furthermore showed the possibilities of using organoid models for drug screening (79).

The results of these studies are of great significance for the field of drug discovery, but also open the possibility of using organoid cultures to study the effects of radiotherapy treatment on normal (or cancer-derived) tissue. Furthermore, 3D culturing models are closer to in vivo tissue response than 2D cell cultures in reaction to exogenous stimuli, such as drugs and irradiation (80, 81). For example, in response to irradiation 2D cell culture models have been shown to be far more sensitive than 3D models or in vivo models (80). Therefore, organoid studies could be of huge importance for studying mechanistic and tissue specific responses to radiation in the future, but may also offer the possibility of personalized treatment planning based on patient derived organoid cultures.

**Developments to optimize radiotherapy**

Current radiobiology studies primarily focus on means to optimize the efficacy of radiotherapy, by increasing the therapeutic window. However, there is no single parameter which can achieve this in the most successful way. Therefore, radiobiological studies are currently looking at many
different parameters that can be changed to achieve an enhanced therapeutic window. Physically, the development of high precision radiation techniques has enabled the delivery of more accurate beam delivery, and therefore a reduction of the dose to the normal tissue surrounding the tissue. Furthermore, a better understanding of both normal tissue responses and signalling pathways altered in cancer (stem cells) has enabled the possibilities of altering the biological responses to radiation, allowing for the specific targeting of tumor cells to yield a more radiosensitive phenotype and/or even protection of normal tissue.

High precision techniques to enhance radiotherapy

High precision techniques, such as intensity modulated radiotherapy (IMRT) and particle therapy, can be used to more accurately deliver radiation to the desired tumor target and spare more normal tissue from irradiation. As discussed above, dose to normal tissue results in side effects (both early and late), and thus the use of IMRT and particle therapy is increasing.

In recent decades, the use of IMRT to treat head and neck cancers has become increasingly more popular (82-85). This is due to the location of critical organs and structures in the head and neck region, such as the brainstem and spinal cord, optic nerves (86) or submandibular and parotid salivary glands (37, 87, 88) which can be spared better using IMRT than conventional radiotherapy practices.

However, although IMRT is intended to reduce radiation-induced side effects by sparing healthy tissue, not all side effects are eliminated. Despite sparing the parotid gland, xerostomia is still a persistent problem even in patients who undergo IMRT (82, 89). Furthermore, somewhat counter-intuitively, IMRT can result in a smaller effect in tissue sparing than might be expected due to a lower dose tolerance of parts of organs adjacent to an area receiving a high dose (88). When critical regions of organs are within the irradiation field a relative increased detrimental effect has been shown at lower doses. This is known as the bath-and-shower effect, and preclinical data indicates that it may affect many important organs, including cervical spinal cord and salivary glands (88, 90-92). Therefore, other high precision techniques such as particle therapy (protons or carbon ions) may be of even greater benefit than IMRT, as exit dose is eliminated and bath doses can be prevented.

Since charged particles were first used in 1954 until the end of 2014 more than 135,000 patients have been treated worldwide, with a rapid increase in both the number of facilities and the number of treated patients per year over the years (93). The potential benefit of using charged particles based on
the Bragg peak in therapeutic radiation treatment was first suggested in 1946 by Robert Wilson (94). Physically, charged particles can be modulated to encompass the whole tumor in a ‘spread-out Bragg peak’ and this is highly advantageous because entrance dose is minimized and exit dose is eliminated, and thus more efficiently spare healthy tissue (95, 96).

Despite the physical advantages of particle therapy, preclinical studies focusing on particle irradiations have so far been limited and thus much of what we know comes from clinical findings. Charged particles are currently being used in ongoing trials for the treatment of liver cancer, breast cancer, brain cancer and head and neck cancers, among others (97). Initial data suggests that proton therapy is extremely promising for the treatment of liver cancer (98) and head and neck cancers (99) with less adverse side effects than IMRT. However, trials are still ongoing, and further preclinical research and clinical trials are required to investigate the long-term side effects of particle therapy.

Preclinical studies of particle radiation generally investigate the differential induction and expression of various pathways important for the biological responses to radiation therapy, such as DNA damage induction and repair (100) or induction of apoptosis (101). Particle irradiation induces a more complex form of DNA damage (known as clustered damage) than X-ray irradiations. Clustered damage consists of two or more lesions in close proximity to each other on a DNA helix, and is usually repaired slower or remains unrepaired, potentially resulting in increased cell death (100). Furthermore, in terms of apoptosis induction, our group has shown that particles with a high linear energy transfer (LET) induce a greater level of p53 serine-37 phosphorylation, a phosphorylation event is involved in the induction of cellular apoptosis (101). Increased cellular death due to unrepaired lesions and induction of apoptosis, combined with the ability to better target the tumor with particles, all support the idea that particle irradiation is of greater strength in tumor control and limiting unwanted side effects than photon irradiation.

Charged particle irradiation is generally compared to high-energy photon irradiation based on its relative biological effectiveness (RBE). The RBE is a ratio of the doses of differing radiation modalities to reach a defined biological endpoint. For proton irradiation, a RBE of 1.1 is generally accepted for comparison with photons (102). This value was calculated based on the analysis of multiple in vivo and in vitro studies (103, 104). However, while RBE is dependent on the differing endpoints that can be used to compare radiation modalities, it is also dependent on many other parameters including the
LET, position in the spread-out Bragg peak and even the tissue/cell lines being studied (102, 105-108), and these parameters were not considered in the calculation of the general RBE for protons. While the RBE is a useful concept for explaining different radiation modalities, it is perhaps not the best way to compare radiations types, as a single RBE can never be determined.

**Drugs and other agents to enhance radiotherapy**

To enhance the beneficial effects of treatment and minimize the detrimental side effects of radiotherapy, radiotherapy treatment is frequently combined with surgery, immunotherapy or chemotherapy. Combining radiotherapy with chemotherapy is not a new approach, and many attempts have been made to take advantage of the multitude of preclinical data acquired in order to enhance radiosensitivity of tumors (via DNA repair pathways or other signal transduction pathways) (109).

Targeting the DNA damage response (DDR) is one of the most common methods of enhancing tumor radiosensitivity (110). Within the DDR, one of the best understood and most common approaches to enhance radiosensitivity involves inhibition of poly (ADP-ribose) polymerase (PARP) (111). If a cell is unable to efficiently cope with DSBs, these lesions are lethal. PARP1 is essential for base excision repair and single strand break repair (112). Following inhibition of PARP, unrepaired radiation-induced single strand breaks will form double strand breaks (DSB) during replication and thus increase the load on DSB repair pathways. BRCA1 and BRCA2 are essential proteins of the homologous recombination pathway of DSB repair, which are frequently mutated in cancers. Therefore, targeting PARP in BRCA1/2 deficient tumors is extremely attractive, as the increased load of unrepairable DSBs induces “synthetic lethality”, and has been shown to be a successful means of increasing tumor radiosensitivity (109, 111, 113, 114).

While the use of drugs to enhance radiosensitivity is a promising means of improving radiotherapy, drugs are also being used and developed to reduce or protect against adverse side effects induced by radiotherapy (109). Both early and late radiation induced side effects can be minimized by drug treatment before, during or after radiotherapy. Radical scavengers have been shown to effectively reduce many early side effects, as they bind with free radicals generated by ionizing radiation and thus reduce competition for oxygen (115). Radical scavenger drugs, such as amifostine, have been shown to have a radioprotective effect in normal tissue and are used clinically (115). Reducing late side
effects by therapeutic agents is less advanced than early side effects. However, targeting inflammation responses, particularly inhibition of TGFβ (116), is of interest in preventing/reducing late side effects. Furthermore, pilocarpine (117, 118), IGF (119, 120) and keratinocyte growth factor (KGF) (121) have been demonstrated to reduce radiation-induced salivary gland loss of function in in vivo models for radiotherapy of head and neck cancers, while early results indicate that ACE inhibition by Captopril may reduce the side effects of irradiation of normal heart and lung during thoracic irradiation (122).

Concluding remarks
While the benefits of radiotherapy greatly outweigh the adverse effects, and our knowledge of radiation treatment is ever-growing, there is still much to be understood about radiotherapy and its effects on normal tissue. There still remain many unanswered radiobiological questions. For instance: How can we best enhance the therapeutic window of radiation treatment? If a particular pathway is modulated to induce death in a tumor, what is the effect in normal tissue? If cell death is also enhanced in the normal tissue, then the overall benefit of modulating this pathway is lost, as there is no increase in the therapeutic index. The development of new models to study radiation-induced side effects and obtaining new knowledge regarding possible drug targets for radiosensitization may further enhance our knowledge.

Aim and outline of the thesis
The overall aim of this thesis is to establish and use new in vitro models to gain more relevant and personalized insight into the response of stem cells (both normal tissue and cancer stem cells) to radiotherapy, which can be used to enhance the overall efficacy of radiotherapy in the future. As discussed above, these new models (organoids) can potentially offer a more patient-specific response, while their 3D nature may represent a response more similar to the real in vivo patient response than the more commonly in vitro models. In this thesis, mechanisms of radioresistance, normal tissue damage and the effects of low dose irradiations on normal tissue stem cells are investigated. In vitro models to study these limitations are developed and possible targets to increase radiosensitivity are identified.

TGF-β signalling has been implicated in radioresistance in many cell lines. Thus, in Chapter 2, we investigate the role of ΔNp73, a truncated form of p73 (a member of the p53 superfamily), in TGF-β signalling.
Targets for increasing tumor radiosensitivity via means of drugs are an invaluable tool in radiation research and therapy. DDR proteins offer an intriguing target for increasing radiosensitivity due to the importance of DNA damage in cell death mechanisms. In Chapter 3, a novel role in the DDR of Hsp70 (a molecular chaperone) and DNAJB6 (a co-chaperone of Hsp70) is investigated, offering a potential new drug target for enhanced radiosensitivity.

Cancer stem cells frequently are associated with an increased resistance to radiotherapy than other cells. In Chapter 4, a previously defined CSC-like subpopulation (CD44+/CD24-) of esophageal cancer cell lines was targeted via the mTOR pathway to reduce radiosensitivity. Furthermore, stimulation of mTOR pathway was investigated in patient derived esophageal cancer material containing this subpopulation.

Radiation therapy using charged particles spares normal tissue to a much greater extent than conventional radiotherapy. However, the effects of irradiations with varying LETs on normal tissue stem cells is not yet known, as in vitro models are not readily available to investigate this. In Chapter 5, a newly developed in vitro model for studying the effects of irradiation on normal tissue stem cells is used to compare conventional photon irradiations with carbon ion irradiations with varying LET in terms of survival post-irradiation.

LDHRS/IRR has been shown in multiple tumor cell lines in the past. However, the clinical relevance of this phenomenon has remained elusive. Chapter 6 focuses on LDHRS/IRR in normal tissue stem cells both in vivo and in vitro. The effect of low dose irradiations is investigated with regard to function and survival of these cells, also with a more clinical fractionated dose.
REFERENCES


102. Paganetti H. Relative biological effectiveness (RBE) values for proton beam therapy. variations as a function of biological endpoint, dose, and linear energy transfer. Physics in Medicine & Biology 2014;59(22):R419.


