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Photon iso-effective dose for cancer treatment with mixed field radiation based on dose–response assessment from human and an animal model: clinical application to boron neutron capture therapy for head and neck cancer

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Abstract

Boron neutron capture therapy (BNCT) is a treatment modality that combines different radiation qualities. Since the severity of biological damage following irradiation depends on the radiation type, a quantity different from absorbed dose is required to explain the effects observed in the clinical BNCT in terms of outcome compared with conventional photon radiation therapy.

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A new approach for calculating photon iso-effective doses in BNCT was introduced previously. The present work extends this model to include information from dose–response assessments in animal models and humans. Parameters of the model were determined for tumour and precancerous tissue using dose–response curves obtained from BNCT and photon studies performed in the hamster cheek pouch *in vivo* models of oral cancer and/or pre-cancer, and from head and neck cancer radiotherapy data with photons. To this end, suitable expressions of the dose-limiting Normal Tissue Complication and Tumour Control Probabilities for the reference radiation and for the mixed field BNCT radiation were developed. Pearson's correlation coefficients and *p*-values showed that *TCP* and *NTCP* models agreed with experimental data (with $r > 0.87$ and *p*-values >0.57).

The photon iso-effective dose model was applied retrospectively to evaluate the dosimetry in tumours and mucosa for head and neck cancer patients treated with BNCT in Finland. Photon iso-effective doses in tumour were lower than those obtained with the standard RBE-weighted model (between 10% to 45%). The results also suggested that the probabilities of tumour control derived from photon iso-effective doses are more adequate to explain the clinical responses than those obtained with the RBE-weighted values. The dosimetry in the mucosa revealed that the photon iso-effective doses were about 30% to 50% higher than the corresponding RBE-weighted values. While the RBE-weighted doses are unable to predict mucosa toxicity, predictions based on the proposed model are compatible with the observed clinical outcome.

The extension of the photon iso-effective dose model has allowed, for the first time, the determination of the photon iso-effective dose for unacceptable complications in the dose-limiting normal tissue. Finally, the formalism developed in this work to compute photon-equivalent doses can be applied to other therapies that combine mixed radiation fields, such as hadron therapy.

Keywords: BNCT, photon iso-effective dose, *TCP*, *NTCP*, head and neck cancer, hamster cheek pouch *in vivo* model of oral cancer

(Some figures may appear in colour only in the online journal)

1. Introduction

Boron neutron capture therapy (BNCT) is a treatment modality for treatment of cancer that involves a tumour selective ^{10}B compound and a specially tuned neutron beam that will allow nuclear interactions to produce short range particles (Locher 1936). In this way, BNCT targets tumour cells with microscopic selectivity while sparing normal tissues from potentially lethal doses of radiation (Hopewell *et al* 2011). After ^{10}B thermal neutron capture, the compound nucleus ^{11}B splits into two high linear energy transfer (LET) particles: an alpha particle and a ^{7}Li ion. A 478 keV gamma-ray is emitted 93.7% of the times by the nuclear decay of ^{7}Li. These high LET light ions deposit their energy in tissue over distances comparable to the size of a mammalian cell, thereby inactivating the neoplastic cells very efficiently, provided that enough reactions are produced within these cells, in DNA or close by. Moreover, since BNCT involves biochemical rather than geometrical radiation targeting, it is also ideally suited to treat undetectable micrometastases (Pozzi *et al* 2012) and foci of malignant transformation in cancerized tissue (e.g. Monti Hughes *et al* 2013).

In addition to the high LET radiation products that result from the thermal neutron capture reaction by ^{10}B, there are other types of radiation that contribute to the total absorbed dose in BNCT. These are intermediate LET protons (produced as a result of the thermal neutron capture reaction by ^{14}N and neutron elastic collision with H nuclei), and low LET gamma-ray (produced as a result of the thermal neutron capture reaction by H and those that contaminate the neutron beam). The severity of biological damage following irradiation depends on the radiation type, the high LET products being more biologically damaging than low LET radiation. Therefore, equal values of absorbed dose of different radiation types do not lead to the same level of biological damage (Burkart *et al* 1999). The dosimetry of BNCT comprises contributions from different types of radiation. Thus, a quantity different from absorbed dose is desirable to explain the effects observed in the clinic of BNCT, to compare different protocols within a BNCT center or between centers, and finally, to optimize the protocols by considering the outcome with BNCT in terms of the outcome with conventional photon radiation.

The standard procedure for computing photon-equivalent doses in BNCT consists in adding the main contributions of the different radiations to the total absorbed dose (i.e. the absorbed doses), each one weighted by a fixed (dose and dose rate independent) factor (Coderre and Morris 1999). The single numbers used in the clinic are the relative biological effectiveness (RBE) and compound biological effectiveness (CBE) factors obtained from cell survival experiments for a given cellular system and for a given endpoint, using ^{60}Co gamma-rays or photons from accelerators as the reference radiation. In this way, each contribution is converted into a term of photon-equivalent dose.

González and Santa Cruz (2012) reported that the standard procedure for computing photon-equivalent doses in BNCT show inconsistencies, which in practice leads to unrealistically high tumour doses. A more suitable approach was then introduced that defines the photon iso-effective dose as the reference dose that produces the same level of cell survival as a given combination of the absorbed dose components of a mixed field BNCT radiation. This approach included first-order repair of sublethal damage and synergistic interactions between the different radiations, exploiting the information obtained from cell survival experiments. In the light of this formalism, the clinical outcome of the treatment of cutaneous melanoma in Argentina (González *et al* 2004, Menéndez *et al* 2009) was assessed against the doses derived from both approaches. As a result, it was found that the previous standard approach was unsuitable to explain the observed outcome, while the number of controlled tumours predicted by the new formalism is statistically consistent with the observed values.

In both animal tumour models and in humans, local tumour control and the response of dose-limiting normal tissues are commonly assessed to evaluate the success of a radiation treatment. Within this context, the photon iso-effective dose formalism has been extended, redefining the photon iso-effective dose as the reference dose that produces the same effect (i.e. tumour control or normal tissue toxicity) as a given combination of the absorbed dose components of BNCT. This new approach allows the determination of the photon iso-effective dose not only for tumours but also for unacceptable complications in the dose-limiting normal tissue.

The hamster cheek pouch oral cancer model has been extensively used as a surrogate model for human oral cancers (Kreimann *et al* 2001a, Vairaktaris *et al* 2008, Chen and Lin 2010, Supsavhad *et al* 2016). Apart from oral cancer studies, this model is also widely accepted as a model of oral toxicity (mucositis) after cancer therapy (Bowen *et al* 2011). Thus, the formalism introduced and the parameters derived from both animal BNCT studies and human photon radiotherapy data were applied retrospectively to evaluate the photon iso-effective doses in head and neck patients treated with BNCT within the clinical trial carried out in Finland (Kankaanranta *et al* 2012). The observed outcomes in patients were assessed against the doses

derived from the standard procedure and the proposed approach to compute photon equivalent doses in BNCT, and compared to the results from photon radiation therapy.

2. Materials and methods

2.1. Formalism

Let $D_1, \ldots, D_n$ be the absorbed dose components of a mixed radiation field. Let D_R be the dose of the reference radiation R. In a previous study (González and Santa Cruz 2012), the iso-effective dose $D_R = D_R(D_1, \ldots, D_n)$ was defined as the reference dose that produces the same cell survival level as a given combination of $D_1, \ldots, D_n$. In other words, let $S = S(D_1, \ldots, D_n)$ denote the survival probability for the combination of the n radiations and $S_R(D_R)$ the survival probability for the reference radiation. Then, the iso-effective dose $D_R = D_R(D_1, \ldots, D_n)$ is the dose of the reference radiation that satisfies

$$S_R(D_R) = S(D_1, \ldots, D_n). \tag{1}$$

If now $D_1, \ldots, D_4$ denote the boron, thermal neutron, fast neutron and gamma absorbed dose components of the mixed field BNCT radiation, and D_R the dose of a photon reference radiation, the solution of equation (1) is the photon dose that produces the same effect to a system (i.e. the same cell survival level) as the combination of the four components in BNCT. In this way, D_R is named/interpreted as a photon iso-effective dose.

For tumours in both animal models and humans, local tumour control is commonly used as an end-point to evaluate the outcome of a radiation treatment (e.g. Kreimann *et al* 2001b, Kato *et al* 2004). Since the probability of local tumour control depends on the surviving fraction of clonogenic tumour cells, the hypothesis behind the iso-effective dose model is that the photon dose D_R that satisfies equation (1) is also the dose that equals the tumour response.

The determination of the photon iso-effective dose in BNCT with equation (1) requires suitable mathematical expressions to describe the survival for the reference photon radiation and for BNCT. These dose–response relationships, described in González and Santa Cruz (2012) using a modified linear quadratic model (MLQ) that accounts for dose-rate dependence of the repair of sublethal (DNA) damage and synergism between different radiations, depend on different parameters that are determined from *in vitro* or *in vivo/in vitro* experiments using a cell line that is considered to represent the tumour under study.

In addition to models of cancer *in vitro*, there are a number of *in vivo* models that have been extensively used in BNCT (Dagrosa *et al* 2007, Pozzi *et al* 2013, Heber *et al* 2014). *In vivo* models better recapitulate the interactions between organs and tissues and the biological pathways involved, and they include the vascular system and its pivotal role in physiology and pathology of tumour growth. Basically, dose–response data including local tumour control and the response of dose-limiting normal tissue can be obtained using these models. Thanks to the incorporation of complexity, results can be extrapolated (albeit with restrictions) to clinical scenarios.

Taking this context into account, the iso-effective dose formalism presented in González and Santa Cruz (2012) has now been extended, redefining the iso-effective dose $D_R = D_R(D_1, \ldots, D_n)$ as the reference dose that produces the same tumour control probability as a given combination of $D_1, \ldots, D_n$.

Let $TCP = TCP(D_1, \ldots, D_n)$ denote the tumour control probability for the combination of the n radiations and $TCP_R = TCP_R(D_R)$ the tumour control probability for the reference radiation. Then, the iso-effective dose $D_R = D_R(D_1, \ldots, D_n)$ is defined as the dose that satisfies

$$TCP_{\mathrm{R}}\left(D_{\mathrm{R}}\right) = TCP\left(D_1, \ldots, D_n\right). \tag{2}$$

If $n = 1, \ldots, 4$ denote the four absorbed dose components of the mixed field BNCT radiation, and R a photon reference radiation, the solution of equation (2) is now the photon dose that produces the same effect to a system (i.e. the same probability of tumour control) as the combination of the components in BNCT.

Note that for models in which the *TCP* is just a function of the survival fraction of cancer cells after radiation treatment (as in the case of mechanistic models obtained from the stochastic models of cell survival), the iso-effective dose that solves equation (2) coincides with the one solving equation (1).

The definition of the iso-effective dose as the reference dose that produces the same local tumour control probability as a given combination of $D_1, \ldots, D_n$ of n radiations can also be extended to dose-limiting normal tissues. Reasoning as before, the goal in this case is to find the reference dose D_{R} that produces the same normal tissue complication probability as the combination of different absorbed dose components of a mixed field radiation. Let $NTCP = NTCP\left(D_1, \ldots, D_n\right)$ and $NTCP_{\mathrm{R}} = NTCP_{\mathrm{R}}\left(D_{\mathrm{R}}\right)$ denote the normal tissue complication probability for the combination of the n radiations and for the reference radiation, respectively. Then, the iso-effective dose $D_{\mathrm{R}} = D_{\mathrm{R}}\left(D_1, \ldots, D_n\right)$ is defined as the dose that satisfies

$$NTCP_{\mathrm{R}}\left(D_{\mathrm{R}}\right) = NTCP\left(D_1, \ldots, D_n\right). \tag{3}$$

2.2. Derivation of photon iso-effective doses in BNCT for head and neck cancer

2.2.1. Tumours. Tumour cure, by definition, requires eradicating all clonogenic tumour cells. Since cell killing by radiation is a stochastic process, the only possible quantitative description is in terms of probability of local tumour control. The probability of local tumour control or *TCP* in radiotherapy is a measure of the probability to locally control a mass of clonogenic cells treated with radiation. At least from the mathematical point of view, a 'tumouricidal' dose cannot be determined and absolute certainty of tumour eradication cannot be achieved.

In Bentzen (1989) and Brenner (1992) a four-parameter *TCP* model,

$$TCP\left(v, D\right) = \mathrm{e}^{-\left(c_1 v^{c_2} \mathrm{S}(D,d)\right)}, \tag{4}$$

was introduced to explain the clinical outcome from a series of patients having recurrent or metastatic malignant melanomas and squamous cell carcinoma of the head and neck. In this model, v represents the tumour volume (in cm^3) with c_1 and c_2 parameters that modulate its effect on local control probability, and $S(D, d) = \exp\left(-D\left(\alpha + \beta d\right)\right)$ is the simplest LQ survival expression that accounts for fractionated regimens with total photon dose D and dose per fraction d. In González and Santa Cruz (2012), a modified version of equation (4) for mixed field radiations was proposed and used to assess if the predicted number of locally controlled tumours was statistically consistent with that observed in the Argentine BNCT treatments of cutaneous melanoma. The formalism was developed under the framework of the dual radiation action theory (TDRA), which proved suitable for explaining the survival experiments that involved sequential or simultaneous exposures to radiations of different quality, such as the case of BNCT (Kellerer and Rossi 1972, Kellerer and Rossi 1978). According to the postulates of the TDRA, the biological system must show synergism, i.e. the additional creation of lethal lesions when sublesions produced by one radiation interact not only among themselves but also with sublesions produced by the other radiations. Therefore, cell killing is described in terms of single radiation events that produce lethal lesions (with a yield proportional to the absorbed dose), and independent radiation events that produce pairs of sublesions that

combine to form a lethal lesions (with a yield proportional to the product of the absorbed doses of the different radiations). Then, the proposed model explicitly includes first-order lesion repair by means of the generalized Lea–Catcheside factor in the LQ survival model (Lea and Catcheside 1942, Lea 1946), and takes into account synergistic interactions between the main different radiations considering additional mixed terms in the survival expression (Zaider *et al* 1980).

In the following paragraphs, the derivation of the photon iso-effective dose expression for tumours of the head and neck based on the *TCP* models for the reference radiation and for the mixed field BNCT radiation mentioned above will be presented.

Let α_{R} and β_{R} be the coefficients of the single fraction LQ survival model for the reference radiation S_{R}, and let $G_{\mathrm{R}}(\theta')$ denote the generalized Lea-Catcheside time factor for the irradiation time θ' (Lea and Catcheside 1942). Then, the proposed expression for TCP_{R} is

$$TCP_{\mathrm{R}}(v, D_{\mathrm{R}}) = \mathrm{e}^{-\left(c_1 v^{c_2} S_{\mathrm{R}}(D_{\mathrm{R}})\right)}, \tag{5}$$

with

$$S_{\mathrm{R}}(D_{\mathrm{R}}) = \mathrm{e}^{\left(-\left(\alpha_{\mathrm{R}} D_{\mathrm{R}} + G_{\mathrm{R}}(\theta')\beta_{\mathrm{R}} D_{\mathrm{R}}^2\right)\right)}. \tag{6}$$

Assuming that the repair kinetics is well described by a bi-exponential decline with fast and slow characteristic repair times $t_{0_{\mathrm{f}}}$ and $t_{0_{\mathrm{s}}}$ independent of LET (Millar and Hopewell 2007, Schmid *et al* 2010), the expression for the Lea-Catcheside time factor is given by

$$G_{\mathrm{R}}(\theta', t_{0_{\mathrm{f}}}, t_{0_{\mathrm{s}}}) = a_{\mathrm{R}_{\mathrm{f}}} G\left(\theta', t_{0_{\mathrm{f}}}\right) + a_{\mathrm{R}_{\mathrm{s}}} G(\theta', t_{0_{\mathrm{s}}}). \tag{7}$$

In equation (8), $a_{\mathrm{R}_{\mathrm{f}}}$ and $a_{\mathrm{R}_{\mathrm{s}}}$ are the proportions of sublesions repaired by the fast and slow kinetics for radiation R (with $a_{\mathrm{R}_{\mathrm{f}}} + a_{\mathrm{R}_{\mathrm{s}}} = 1$), and

$$G(\theta',\ t_0) = \frac{2t_0}{\theta'}\left(1 - \frac{t_0}{\theta'}\left(1 - \mathrm{e}^{-\frac{\theta'}{t_0}}\right)\right) \tag{8}$$

is the time factor for simultaneous build up and repair of radiation damage, considering an irradiation at a constant dose rate.

Allowing the different components of the mixed field BNCT radiation to act synergistically (i.e. sublesions produced by different radiations combine to form lethal lesions, (Zaider and Rossi 1980)), the tumour control probability for the combination of 4 radiations is

$$TCP(v, D_1, \ldots, D_4) = \mathrm{e}^{-\left(c_1^* v^{c_2^*} S(D_1,\ldots,D_4)\right)}, \tag{9}$$

with

$$-\ln\left(S(D_1, \ldots, D_4)\right) = \sum_{i=1}^{4} \alpha_i D_i + \sum_{i=1}^{4}\sum_{j=1}^{4} G_{ij}(\theta)\sqrt{\beta_i \beta_j} D_i D_j, \tag{10}$$

where α_i and β_i are the coefficients of the single fraction LQ model, and $G_{ij}(\theta)$ the time factor reported by Suzuki (1998) for a simultaneous mixed irradiation that accounts for the repair of pairs of sublesions produced by radiations i and j during the irradiation time θ. If production of one of the sublesions does not affect the production of the other one, it can be shown that $G_{ij}(\theta)$ can be expressed as a sum of independent G factors (equation (8)), i.e.$G_{ij}(\theta) = a_i G_i(\theta) + a_j G_j(\theta)$, each one weighted by the relative proportion between components a_i and a_j, with $a_i + a_j = 1$.

The tumour control probabilities given by equations (5) and (9) are now used in equation (2). Then, the photon iso-effective dose $D_{\mathrm{R}} = D_{\mathrm{R}}\left(D_1, \ldots, D_4\right)$ is obtained by solving

$$\mathrm{e}^{-(c_1 v^{c_2} S_{\mathrm{R}}(D_{\mathrm{R}}))} = \mathrm{e}^{-\left(c_1^* v^{c_2^*} S(D_1,\ldots,D_4)\right)}. \tag{11}$$

Equation (11) must be numerically solved to obtain D_{R}. However, if the dose–response experiments are carried out at a constant irradiation time (i.e. $G_{\mathrm{R}}\left(\theta'\right) = G_{\mathrm{R}}$ is constant) or if variations of $G_{\mathrm{R}}\left(\theta'\right)$ during irradiation can be neglected (i.e. G_{R} is approximately constant for the different irradiation times), equation (11) can be solved for D_{R},

$$D_{\mathrm{R}}\left(D_1, \ldots, D_4\right) = \frac{1}{2}\frac{(\alpha/\beta)_{\mathrm{R}}}{G_{\mathrm{R}}}\left(\sqrt{1 + \frac{4G_{\mathrm{R}}}{\alpha_{\mathrm{R}}(\alpha/\beta)_{\mathrm{R}}}\left(\ln\left(\frac{c_1^*}{c_1} v^{c_2^* - c_2}\right) + \sum_{i=1}^{4}\alpha_i D_i + \sum_{i=1}^{4}\sum_{j=1}^{4} G_{ij}(\theta)\sqrt{\beta_i\beta_j} D_i D_j\right)} - 1\right). \tag{12}$$

Equation (12) is the general expression to calculate the photon iso-effective dose for a tumour exposed to the mixed-LET BNCT field radiation. Note that if the population of target tumour cells to obtain the same probability of local tumour control is equal for both radiation treatments (i.e. $c_1 = c_1^*$ and $c_2 = c_2^*$), then equation (12) is also the solution of equation (1).

The photon iso-effective tumour dose given by equation (12) depends on the radiobiological parameters that characterize the *TCP* models for the photon reference radiation and for BNCT. Sections 2.3 and 2.4 describe the experimental data obtained from a small animal tumour model and the methodology used to obtain the corresponding parameters for head and neck in patients.

2.2.2. Dose-limiting normal tissues. The acute effect of concern when treating head and neck cancer with radiation is oral mucositis grade 3 or higher (⩾G3) caused by the depletion of mucosa cells (Monti Hughes *et al* 2015b).

Strigari *et al* (2012) proposed a *NTCP* model able to predict mucositis ⩾G3 after head and neck cancer radiotherapy with photons, for standard and altered schedules of dose delivery and overall treatment time. Essentially, the normal tissue complication probability as a function of the total dose D and overall treatment time T is calculated by the integral of a normal distribution

$$NTCP\left(D, T\right) = \frac{1}{\sqrt{2\pi}}\int_{-\infty}^{s}\exp\left(-\frac{t^2}{2}\right)\mathrm{d}t, \tag{13}$$

where

$$s = \frac{EQD_2(D) - TD_{50}(T)}{m \cdot TD_{50}(T)}.$$

In equation (13), $\alpha/\beta = 10\,\mathrm{Gy}$ and $EQD_2 = \frac{D\ (1+d/(\alpha/\beta))}{1+2/(\alpha/\beta)}$ is the total dose which, delivered in fractions of 2 Gy, is biologically equivalent to the total dose D given in dose fractions of size d. The parameters m and $TD_{50}\left(T\right)$ are the slope of the *NTCP* versus dose curve and the tolerance dose for a complication probability of 0.5, respectively. In particular, the value of m and the tolerance dose dependency with the overall treatment time (in days) were taken from data of acute rectal toxicity after prostate cancer radiotherapy (see Strigari *et al* 2012).

In the current study, equation (13) is rewritten in terms of the single-fraction photon reference dose D_{R} as

$$NTCP\left(D_{\mathrm{R}},\ T=1\right)=\frac{1}{\sqrt{2\pi}}\int_{-\infty}^{s'}\exp\left(-\frac{t^2}{2}\right)\mathrm{d}t, \tag{14}$$

where

$$s'=\frac{\frac{D_{\mathrm{R}}\left(1+\frac{D_{\mathrm{R}}\,G_{\mathrm{R}}(\theta')}{(\alpha/\beta)_{\mathrm{R}}}\right)}{\left(1+\frac{2\,G_{\mathrm{R}}(\theta')}{(\alpha/\beta)_{\mathrm{R}}}\right)}-TD_{50}(T=1)}{m\cdot TD_{50}(T=1)}.$$

Based on the evidence that the behavior of cells of rectal and oral/pharyngeal mucosa is similar, values of $(\alpha/\beta)_{\mathrm{R}}$, m and $TD_{50}\ (T=1)$ equal to 10 Gy, 0.17 and 39.8 Gy, respectively, were taken or calculated from Strigari's work. The time factor G_{R} in this case was computed as in equation (7) for fast and slow characteristic repair times $t_{0_{\mathrm{f}}}=27/\ln 2$ min and $t_{0_{\mathrm{s}}}=150/\ln 2$ min, reported in Stüben *et al* (1997) for mouse lip mucosa.

Strigari's *NTCP* model assumes the validity of the EQD_2 concept which is based on the linear quadratic model of radiation effect. Essentially, assuming that the biological effect E of a dose D split into n fractions of d dose each is described by $E=-\ln(S)=\alpha D\ \left(1+d/\left(\alpha/\beta\right)\right)$, the EQD_2 formula is derived making the biological effect E for the dose D and fraction size d equal to the dose EQD_2 and fraction size 2 Gy. Following the same line of thought of making effects equal, and assuming as in the case of tumours that the different components in BNCT act synergistically in normal tissues to cause the effect, the photon iso-effective dose $D_{\mathrm{R}}=D_{\mathrm{R}}\left(D_1,\ldots,D_4\right)$ for mucosa must satisfy

$$-\ln\left(S_{\mathrm{R}}\left(D_{\mathrm{R}}\right)\right)=-\ln\left(S\left(D_1,\ldots,D_4\right)\right). \tag{15}$$

This means that $D_{\mathrm{R}}=D_{\mathrm{R}}\left(D_1,\ldots,D_4\right)$ is described as in equation (12), but without the logarithmic term:

$$D_R\left(D_1,\ldots,D_4\right)=\frac{1}{2}\frac{\left(\alpha/\beta\right)_{\mathrm{R}}}{G_{\mathrm{R}}}\left(\sqrt{1+\frac{4G_{\mathrm{R}}}{\alpha_{\mathrm{R}}\left(\alpha/\beta\right)_{\mathrm{R}}}\left(\sum_{i=1}^{4}\alpha_i D_i+\sum_{i=1}^{4}\sum_{j=1}^{4}G_{ij}\left(\theta\right)\sqrt{\beta_i\beta_j}D_iD_j\right)}-1\right). \tag{16}$$

Therefore, the complication probability after a BNCT irradiation $NTCP=NTCP\left(D_1,\ldots,D_4\right)$ is obtained by replacing D_{R} in expression (14) by $D_{\mathrm{R}}=D_{\mathrm{R}}\left(D_1,\ldots,D_4\right)$.

The photon iso-effective dose for mucosa depends on the radiobiological parameters of the different radiations involved in BNCT. The determination of these parameters using dose–response assessment in an *in vivo* animal model (section 2.3) and the *NTCP* models presented above are described in section 2.4.

2.3. In vivo animal model and experiments

2.3.1. Hamster cheek pouch oral cancer and pre-cancer models. The chemical carcinogenesis model in the hamster cheek pouch is the most widely accepted model of oral cancer (Chen and Lin 2010, Supsavhad *et al* 2016). The mode of cancerization in this model mimics the mode of action of tobacco and alcohol, the two most recognized oral carcinogens in humans (Nagini *et al* 2009). Essentially, carcinogenesis protocols induce premalignant and malignant changes that closely resemble spontaneous human oral mucosa lesions and mimic the spontaneous process of malignant transformation in the human oral mucosa (Kreimann *et al* 2001a, Vairaktaris *et al* 2008, Monti Hughes *et al* 2015a), inducing tumours surrounded by precancerous tissue.

The hamster cheek pouch model of oral cancer was extensively used to explore new applications of BNCT, study its radiobiology and improve its therapeutic efficacy (e.g. Kreimann *et al* 2001b, Trivillin *et al* 2006, Pozzi *et al* 2009, Molinari *et al* 2012).

Local tumour control studies were performed using the classical carcinogenesis protocol (Kreimann *et al* 2001a) that involves topical application of dimethylbutylamine (DMBA) in the hamster cheek pouch 2–3 times a week for 12 weeks. However, this carcinogenesis protocol is overly aggressive, giving rise to a radiosensitive precancerous tissue that is not suited to evaluating normal tissue toxicity.

In a clinical scenario, confluent oral mucositis is a frequent, dose-limiting side effect during conventional radiotherapy (Jensen and Peterson 2014) and is an important consideration in BNCT for advanced head and neck cancers (Kankaanranta *et al* 2012, Wang *et al* 2014). Thus, a second model that mimics human oral carcinogenesis more closely than the classical carcinogenesis protocol was used to assess radiotoxicity in terms of the induction of mucositis in precancerous tissue. The model of oral pre-cancer involves topical application of DMBA, twice a week, for 6 weeks. The reduction in the number of applications of DMBA leads to the development of a less aggressive, and less radiosensitive, precancerous tissue (Heber *et al* 2010) that is more appropriate to study radiotoxicity.

2.3.2. Tumour control studies in the oral cancer model. The right cheek pouches of young non-inbred Syrian hamsters received a topical application of 0.5% DMBA in mineral oil, twice a week, for 12 weeks. The treated pouch was periodically everted under light ketamine-xylazine anesthesia and examined to monitor tumour development. Once the exophytic tumours had developed and reached a diameter of approximately $\geqslant$1 mm, the animals were irradiated for BNCT studies (Molinari *et al* 2011). All the cancerized animals developed a variable amount of tumours amenable to irradiation within the first month after completion of the carcinogenesis protocol (Heber *et al* 2010). The tumour-bearing pouches were exposed to: (1) Photon irradiation (*R*) (this study); (2) neutron beam only irradiation (BO) (this study); and (3) the neutron beam in the presence of the boron compound L-boronphenylalanine fructose or L-BPA-F (BPA-BNCT) (previous studies: Pozzi *et al* 2009, Molinari *et al* 2011, 2012, Monti Hughes *et al* 2016).

Photon irradiations were performed at Angel H Roffo Institute of Oncology, in Buenos Aires, using a special device that allows the selective irradiation of the cheek pouch following eversion onto a protruding shelf. Animals received gamma radiation from a ^{60}Co source, which was then replaced by a 6MV Linear Accelerator source.

BPA-BNCT and BO groups were irradiated at the thermal column central facility (FCCT) in the RA-3 nuclear reactor (Buenos Aires). A lithium-6 carbonate shielding was used to protect the body of the animal while the tumour bearing cheek pouch was everted out of the enclosure onto a protruding shelf for exposure (Monti Hughes *et al* 2013). Based on previous studies (e.g. Kreimann *et al* 2001a, 2001b), boron concentrations of 30 and 15 ppm in tumour and precancerous tissue, respectively, were considered for dose calculation.

2.3.3. Mucositis studies in an oral pre-cancer model. The right cheek pouches of young non-inbred Syrian hamsters received a topical application of 0.5% DMBA in mineral oil, twice a week, for 6 weeks (Heber *et al* 2010). One week after the end of carcinogenesis protocol, the cheek pouches were exposed to: (1) neutron beam only irradiation (BO) (Monti Hughes *et al* 2011, 2013 and this study); and (2) the neutron beam in the presence of the boron compound L-BPA-F (BPA-BNCT) (previous studies: Monti Hughes *et al* 2011, 2013, 2015b). BPA-BNCT and BO groups were irradiated at the thermal column central facility following the same procedures for the local tumour control studies.

For both models, L-BPA-F was administered intravenously [iv] at a total dose of 15.5 mg ^{10}B/kg body weight (b.w.). The animals were irradiated 3 h post injection. Irradiations, iv injections and follow up were performed under intraperitoneal ketamine-xylazine-anesthesia.

2.3.4. Follow up. All animals were followed for one month after irradiation. The tumour and precancerous tissue responses were assessed by visual inspection and tumour volume assays were performed before irradiation and at 7, 14, 21 and 28 d post-irradiation. The therapeutic effect of all irradiation protocols was evaluated on those tumours that were present at the time of irradiation. Only exophytic tumours that reached a volume of $\geqslant 1\,\mathrm{mm}^3$ and $\geqslant 0.7\,\mathrm{mm}$ in thickness and were not localized in regions of precancerous tissue affected by necrosis were considered for evaluation. For the purpose of this study, final tumour responses at 28 d post-treatment were considered and were binarized as complete response (CR: disappearance of the tumour on visual inspection) and other response (OR: partial response (PR) + no response (NR)). The response categories CR, PR and NR have been previously defined and used as endpoints in previous BNCT studies by our group (Trivillin *et al* 2006, Heber *et al* 2014).

The severity of mucositis was evaluated semi-quantitatively in precancerous tissue using a 6 point scale (Grade 0–5) as previously described (Monti Hughes *et al* 2015b). Grading was based on the most severe macroscopic feature. As in Alvarez *et al* (2003), Strigari *et al* (2012) and Wu *et al* (2012), mucositis grade 3 or higher was considered clinically relevant.

Animal experiments were carried out in accordance with the Guidelines laid down by the National Institute of Health in the USA regarding the care and use of animals for experimental procedures and in accordance with protocols approved by the National Atomic Energy Commission Animal Care and Use Committee (CICUAL-CNEA).

2.4. Parameters of the iso-effective dose models from dose–response data

2.4.1. Head and neck tumours. As explained above, tumour response data to (1) the photon reference radiation; (2) the neutron beam only; and (3) the neutron beam in the presence of the boron compound L-BPA-F were obtained for the *in vivo* oral cancer model in the hamster cheek pouch. These three sets of data were then used to estimate the parameters of equation (12) as follows.

2.4.1.1. Reference radiation parameters. The TCP_{R} model given by equation (5) depends on 4 parameters: c_1 and c_2 (related with the tumour volume), and the coefficients α_{R} and β_{R} of the survival model for the reference radiation S_{R}. The fast and slow characteristic repair times $t_{0_{\mathrm{f}}}$ and $t_{0_{\mathrm{s}}}$ and the proportions of the sublesions repaired by the two kinetics $a_{R_{\mathrm{f}}}$ and $a_{R_{\mathrm{s}}}$ for the reference low LET radiation are taken as 24/ln2 min and 14/ln2 h, and 0.53 and 0.47, respectively, following the results reported by Schmid *et al* (2010) for a squamous cell carcinoma cell line.

Thames *et al* (1989) reported that, with some exceptions, α/β values for human tumours are high and similar to those observed for early-reacting normal tissues, also in agreement with studies in rodents. Thus, in accordance with these authors and Bentzen *et al* (1991) for tumours in the oral cavity and oropharynx, a $\alpha_{\mathrm{R}}/\beta_{\mathrm{R}} = 10$ Gy was assumed. In this way, the number of the free model parameters in equation (5) was reduced by one.

The maximum likelihood estimation method (mle) was then used to determine the parameters of the TCP_{R} model (i.e. c_1, c_2 and α_{R}) that maximize the probability of the observed tumour response to the photon reference radiation. Prediction bounds of the model and the confidence interval of the coefficients (all for 1STD) were obtained by parametric bootstrapping as follows. The response of each lesion was sampled using the tumor control probability TCP_{R} with the estimated parameters. For every set of simulated responses, the corresponding model coefficients were obtained by mle. This procedure was repeated 10 000 times and, from the resulting sampling distribution of the parameters, confidence intervals were computed. Prediction bounds of the model were finally determined using the TCP_{R} curves generated by all the sampled sets of coefficients.

The agreement of the model prediction to observed tumour responses was assessed in two ways. First, tumours were grouped in subsets according to doses and, for each subset, the expected and observed number of positive responses were computed. Then, the Pearson's correlation coefficient between expected and observed data was determined. Second, the p-value for the TCP_R model with the estimated parameters was computed to evaluate if the difference between the observed number of controlled tumours and the expected number according to the model can be due to the randomness of the process. In other words, the null hypothesis tested was that the number of controlled lesions follows the distribution predicted by the TCP_R model.

2.4.1.2. BNCT radiation parameters. Following the methodology and considerations presented in González and Santa Cruz (2012), $S(D_1, \ldots, D_4)$ was expressed as a function of the total absorbed dose $D_T = \sum_{i=1}^{4} D_i$ and the relative contribution of each dose component $f_i = D_i/D_T$

$$-\ln\left(S\left(D_1, \ldots, D_4\right)\right) = \left(\sum_{i=1}^{4} \alpha_i f_i + \sum_{i=1}^{4}\sum_{j=1}^{4} G_{ij}\left(\theta\right)\sqrt{\beta_i \beta_j} f_i f_j\right) D_T^2, \tag{17}$$

where the coefficients for thermal and fast neutrons were assumed the same (i.e. $\alpha_2 = \alpha_3$ and $\beta_2 = \beta_3$), and the photon coefficients of the beam were assumed to be equal to those of the reference photons (i.e. $\alpha_4 = \alpha_R$ and $\beta_4 = \beta_R$). In expressions G_{ij}, the proportions of the sublesions repaired by the two kinetics a_{i_f} and a_{i_s} were taken as 0.57 and 0.43 for the low LET radiation (i.e. for $i = 4$), and 0.2 and 0.8, for the high LET radiations (i.e. for $i = 1, 2, 3$) (Schmid *et al* 2010).

Expression (17) was considered in equation (9) to obtain the tumour control probability for the combination of the four BNCT components. Therefore, the original *TCP* model was reduced to a six-parameter expression. In order to further reduce the number of free parameters, target tumour cells were considered the same, regardless of the irradiation protocol. Then, the volume parameters of the *TCP* c_1^* and c_2^*, were equal to c_1 and c_2, respectively, the model free parameters being thus reduced to four (i.e. the boron and neutron coefficients $\alpha_1,\ \beta_1, \alpha_2, \beta_2$).

Based on the data from the tumour control studies in the oral cancer model, the mle method was applied to determine the *TCP* model parameters that best predict the tumour response to the neutron beam only and to the neutron beam in the presence of L-BPA-F. Note that the (neutron + L-BPA-F)-based data contain information not only on the effect of ^{10}B neutron capture reactions, but also on the neutron reactions with the hydrogen and nitrogen present in tissues. As in the case of the reference radiation, prediction bounds of the model and the confidence interval of the coefficients (all for 1STD) were obtained by parametric bootstrapping. Also, the Pearson's correlation coefficient between expected and observed number of controlled tumours and p-values for the same null hypothesis considered above in the case of the reference radiation were computed for the *TCP* model. The whole set of experimental data and also two separate subsets corresponding to BO and BNCT data were considered in the calculations.

2.4.2. Mucosa.

2.4.2.1. Reference radiation parameters. As shown in section 2.2.2, the proposed *NTCP* model for acute mucosa toxicity ⩾Grade 3 given a photon dose D_R depends on the coefficients α/β, m and TD_{50} $(T = 1)$. These parameters were directly taken (i.e. for α, α/β and m) or calculated for a single fraction of dose delivery from Strigari's work. Therefore, expression

Table 1. Radiobiological parameters of the *NTCP* model for the reference radiation taken (i.e. for α/β and m) or calculated for a single fraction of dose delivery from Strigari *et al* (2012).

NTCP			
m	TD_{50} (Gy)	Alpha (Gy^{-1})	Beta (Gy^{-2})
0.17	39.8	0.35	0.035

(14) with values reported in table 1 is the final *NTCP* model for single fraction photon irradiations used in this work.

2.4.2.2. BNCT radiation parameters. The normal tissue complication probability $NTCP = NTCP\,(D_1, \ldots, D_4)$ given by equations (14) and (16) depends on eight radiobiological parameters that were reduced, following the same considerations as for tumours, to four. This means that the coefficients for thermal and fast neutrons were assumed the same, and the photon coefficients of the BNCT beam were assumed to be equal to those of the reference photons. In particular, an $\alpha_R = 0.35\ Gy^{-1}$ and an $(\alpha/\beta)_R = 10$ Gy were taken from table 1.

Based on the data from the mucositis studies in the oral pre-cancer model, the mle method was again applied to determine the free parameters of the *NTCP* model that best predict the mucosa response. Prediction bounds of the model and confidence intervals of the estimates were obtained by bootstrapping. The agreement of the model prediction to observed number of animals with mucositis G3 or greater was assessed in the same two ways as for tumours. The null hypothesis tested in this case for the calculation of the p-value was that the number of animals that developed mucosa toxicity G3 or higher follows the distribution predicted by the *NTCP* model.

2.5. Examples of application in the clinic of BNCT for recurrent head and neck cancer

From December 2003 through September 2008, 30 patients with inoperable, locally recurred head and neck cancer were treated with BPA-mediated BNCT in a single-center Phase I/II study carried out in Finland (Kankaanranta *et al* 2012). Most of the patients received two treatments administered at 3- to 5- weeks intervals, using the Finnish BNCT facility of the FiR 1 250 kW Triga Mark II nuclear reactor. An L-BPA-fructose dose of 400 mg kg^{-1} was administered intravenously over a period of 2 h before neutron irradiation. Blood samples collected periodically after the infusion of the boron compound were analyzed to estimate boron concentrations as a function of time. The patients were followed at 4- to 12- weeks intervals after BNCT, and CT or MRI studies were performed 1, 3, 6, and 12 months after irradiation. Tumour response was assessed with the RECIST (response evaluation criteria in solid tumours) guideline, and adverse effects were evaluated according to the National Cancer Institute common terminology criteria version 3.0. The disease was considered stable if a minimum of 3 months (calculated from the date of the first BNCT fraction) elapsed without progression. Results of the locoregional failure-free survival, progression-free survival, and overall survival after BNCT for the 30 patients treated within this trial can be found in Kankaanranta *et al* (2012).

In this work, the photon iso-effective dose formalism with the estimated parameters from the *in vivo* oral cancer model and human data was applied retrospectively to evaluate the tumour and dose-limiting normal tissue doses for head and neck patients treated in Finland.

Photon iso-effective doses to the gross tumour volume (GTV) and to the mucosa tissue were computed using the whole blood average boron concentration during irradiations and assuming tumour-to-blood and mucosa-to-blood ratios of 3.5:1 and 2:1, respectively (Kankaanranta *et al* 2012). As mentioned above, the patients received two BNCT applications (with 3–5 weeks interval). Then, tumour doses were computed for each application separately and for the combination of both. For the latter, the effect on the tumor was assumed additive.

Mucositis after the first BNCT irradiation is not influenced by a previous reaction as would occur with mucositis after the second irradiation session. Then, the dose of interest in this work was the maximum value administered after the first BNCT application (i.e. the point corresponding to the prescription dose). Standard photon-equivalent doses obtained using conventional RBE/CBE factors in BNCT (Kankaanranta *et al* 2012) were also computed in tumours and mucosa for comparison purposes. In addition, total absorbed doses for both tissues were reported for reference.

In Provenzano *et al* (unpublished), a *TCP* model for non-uniform doses was constructed following González and Carando (2008) and considering equations (5) and (9). Based on this model and the total dose distributions in the GTV, tumour control probabilities were obtained for both the standard and photon iso-effective models of dose calculation. Results were analyzed in the light of the assessed tumour responses. Finally, normal tissue complication probabilities were estimated also for both models of dose calculation and correlated with the observed radiation toxicity after the first delivered treatment.

3. Results

3.1. Dose–response experimental data and model predictions

Figure 1 shows the observed and expected dose–response curves for the photon reference radiation, the neutron beam only, and the neutron beam in the presence of L-BPA-F obtained from the tumour control studies carried out in the hamster cheek pouch model of oral cancer. For the purpose of displaying the experimental dose–response relationship, a tumour-size corrected dose was computed and assigned to each treated lesion, using the average volume of all the assessed tumours of 0.02 cm^3, as the reference volume (see appendix in Bentzen *et al* (1989)).

Figure 2 shows the corresponding dose–response curves for acute mucosa toxicity G3 or greater obtained from the HN cancer radiotherapy in humans (photons) and from the mucositis studies in the oral pre-cancer model (BO and BPA-BNCT).

The radiobiological parameters of the *TCP* and *NTCP* models for the reference radiation and the combination of the four BNCT components (equations (5), (9), (14) and (16), respectively) are presented in table 2. Based on the calculation of the Pearson's correlation coefficients (r) and the p-values for the *TCP* models (with values of $r = 0.87$ and $r = 0.95$ and p-values of 1 and >0.57, for the reference radiation and for BNCT, respectively), it turned out that the proposed expressions with the estimated parameters are very good candidates to describe the experimental data. The same held for the *NTCP* model for BNCT with the estimated parameters (with $r = 0.99$ and a p-value of 1).

Table 2 shows that the beta parameters of the neutron and boron absorbed dose components for both probability models are much smaller than their corresponding alpha parameters. These findings are in agreement with the radiobiological parameters obtained from *in vitro* cell survival experiments carried out recently in our group for a human UTSCC cell line (unpublished data). The fact that beta parameters for HN cells are very small would imply that the cell killing is mainly due to lethal lesions that arise from the direct action of single

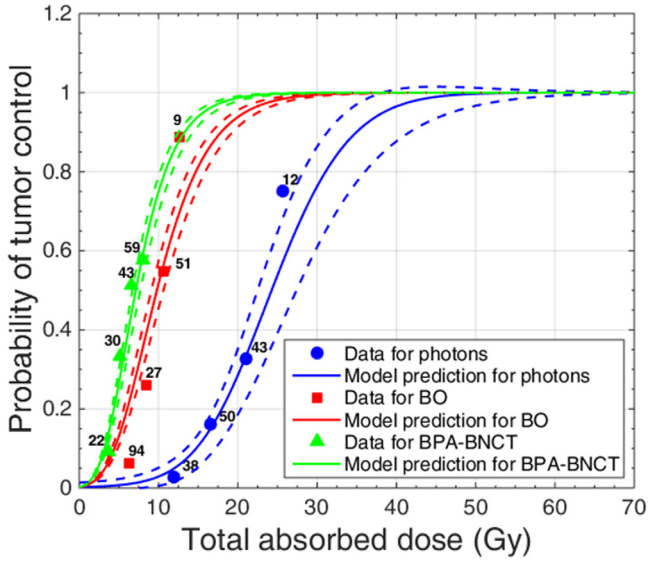

Figure 1. Dose–response curves with prediction bounds for a 1STD confidence level obtained from the tumour control studies carried out in the the hamster cheek pouch model of oral cancer (BO and BPA-BNCT indicate neutron beam irradiations without and in the presence of the boron compound, respectively). *TCP* curves were plotted for a tumour volume of 0.02 cm^3 (average value of 480 assessed tumours). Tumour-size corrected doses were used to display the experimental dose–response relationships. The number besides each point indicates the number of lesions that contribute to the empirical response rate in each dose bin.

events rather than from the incoherent action of two independent events that combine together to produce a lethal lesion. From a mathematical view point, this means that the quadratic and mixed terms involving neutron and boron components could be neglected in equation (10), thus leaving only one term proportional to the square of the dose (i.e. the quadratic term for photons) in the photon iso-effective dose expression (12). Note that for other types of tumor such as cutaneous melanomas, the beta coefficients for all BNCT components are significant (about one tenth of alpha coefficients; see table 3 in González and Santa Cruz (2012)). In such cases, none of the quadratic and mixed terms can be neglected, particularly when high single doses are delivered.

Finally, it is worth mentioning that even if only the low-LET component is considered to have a quadratic dependence with the dose, equation (12) cannot be reduced to the sum of each absorbed dose multiplied by fixed RBE or CBE factors, as proposed by the standard model of dose calculation in BNCT.

3.2. Application examples in the clinic of BNCT for recurrent head and neck cancer

Table 3 shows the general characteristics of the HN patients treated in Finland selected to evaluate tumour doses and their corresponding responses. In particular, these patients had received photon radiotherapy before BNCT (delivered full doses of 66 Gy, 50 Gy and 56 Gy, for patient #1 to #3, respectively).

Table 4 summarizes the dosimetry results obtained for the tumour region (GTV) of these patients. Comparing RBE-weighted and photon iso-effective doses it follows that the standard

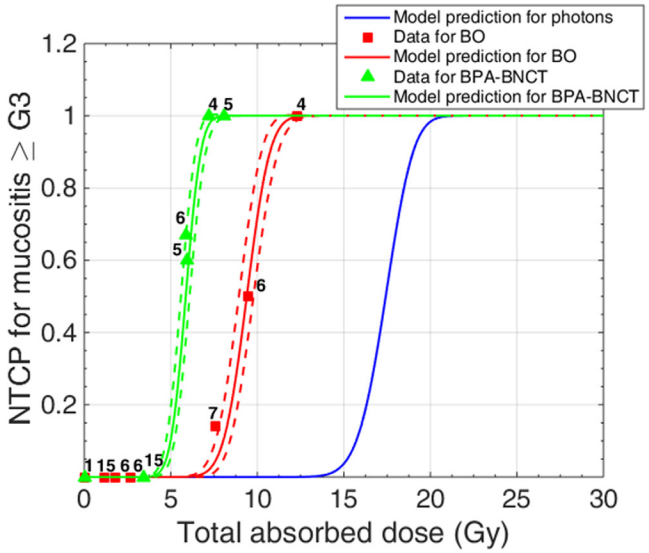

Figure 2. Dose–response curves obtained from the HN cancer radiotherapy in humans (photons) and from the mucositis studies in the oral pre-cancer model (BO and BPA-BNCT indicate neutron beam irradiations without and in the presence of the boron compound, respectively). 1STD prediction bounds are depicted for the latter. The number beside each point indicates the number of lesions that contribute to the empirical response rate in each dose bin.

Table 2. Radiobiological parameters of the *TCP* and *NTCP* models for the reference radiation and for BNCT based on the *in vivo* oral cancer model and human data. One standard deviation is shown only for the coefficients determined in this work.

	TCP		*NTCP*	
	$c_1 = 22 \pm 19$		$m = 0.17$[a]	
	$c_2 = 0.33 \pm 0.09$		$TD_{50} = 39.8$ Gy[a]	
	Alpha (Gy^{-1})	Beta (Gy^{-2})	Alpha (Gy^{-1})	Beta (Gy^{-2})
^{60}Co or 6MV LINAC reference radiation	0.029 ± 0.008	0.0029 ± 0.0008	0.35[a]	0.035[a]
FCCT (RA-3 reactor)				
Beam gamma photons	0.029 ± 0.008	0.0029 ± 0.0008	0.35[a]	0.035[a]
Neutrons	0.37 ± 0.03	$8.8 \times 10^{-9} \pm 2.2 \times 10^{-9}$	2.47 ± 0.03	$1.0 \times 10^{-16} \pm 2.0 \times 10^{-17}$
Boron (BPA)	0.34 ± 0.03	$8.3 \times 10^{-9} \pm 3.1 \times 10^{-9}$	3.09 ± 0.03	$7.0 \times 10^{-17} \pm 2.1 \times 10^{-17}$

[a] Coefficients from table 1.

procedure derives values that are in general higher or much higher than the ones obtained with the iso-effective dose formalism.

Interestingly, minimum doses in the tumour that correspond to the lowest total absorbed doses within the GTV are however quite similar, the RBE-weighted values being sometimes even lower than iso-effective doses. These results are remarkably similar to those obtained in glioblastomas and melanomas with the first version of the photon iso-effective dose formalism

Table 3. Selected patients with inoperable, locally recurred head and neck cancer treated with BPA-mediated BNCT in Finland (Kankaanranta *et al* 2012).

Patient	cancer Type	Nr. BNCT	Time between fractions (weeks)	Tumour (GTV) (cm^3)	Tumour response
#1	SCC	2	3	14	CR
#2	SCC	2	3	83	SD
#3	SCC	2	3	116	SD

SCC: squamous cell carcinoma; CR: complete response; SD: stable disease.

Table 4. Results obtained for the tumour (GTV) for three recurrent HN patients treated in Finland (Kankaanranta *et al* 2012).

		Dose mean (Min, Max)				
Patient	^{10}B Conc. (ppm)	Absorbed (Gy)	RBE-weighted (Gy-Eq)	Photon iso-eff. (Gy(IsoE))	TCP RBE-weighted versus Phot. iso-eff. dose	Observed response
#1 (Fr1)	65	8.5 (6.5, 9.3)	30 (24, 32)		—	—
#1 (Fr2)	55	7.6 (5.4, 8.4)	26 (20, 28)		—	
#1(Comb.)		16.1 (11.9, 17.7)	55 (45, 59)	38 (34, 39)	0.98 versus 0.62	CR
#2 (Fr1)	57	5.7 (4.0, 7.7)	19 (14, 26)		—	—
#2 (Fr2)	47	5.7 (3.8, 7.6)	18 (13, 24)		—	
#2(Comb.)		11.4 (7.8, 15.3)	37 (28, 50)	30 (25, 35)	0.21 versus 0.01	SD
#3 (Fr1)	64	9.9 (4.4, 13.9)	33 (15, 47)		—	—
#3 (Fr2)	58	9.4 (4.6, 12.9)	32 (17, 44)		—	
#3(Comb.)		19.3 (9.0, 26.8)	65 (33, 91)	41 (28, 50)	0.75 versus 0.26	SD

^{10}B Conc. in tumour (3.5 × blood); Fr1 and Fr2: first and second BNCT, Comb: combination of first and second BNCT; CR: complete response; SD: stable disease.

based on radiobiological parameters derived from cell survival assays (González and Santa Cruz 2012). The fact that the photon iso-effective formalism based on experimental data of different nature (i.e. *in vitro* cell survival and *in vivo* tumour control) leads to the same conclusions reinforces the previous assertion that RBE-weighted tumour doses usually overestimate the real photon equivalent values.

The tumour control probabilities obtained considering the whole dose distribution in the GTV for both the standard and photon iso-effective models of dose calculation are shown in table 4 together with the assessed tumour responses. From these results, the most likely values of controlled tumours were computed. It turned out that for the iso-effective dose formalism this value is 1, which coincides with the observed result. The RBE-weighted model derives a most likely value of 2 and assigns a probability of 0.8 to a number of controlled tumours of 2 or more. This is not conclusive and the inclusion of more clinical cases is warranted, but this analysis suggests that the photon iso-effective doses are more adequate to explain the observed tumour responses than the RBE weighted doses.

Different protocols with photons of previously irradiated recurrent head and neck cancer are found in literature, including both single-fraction stereotactic body radiation therapy (S-SBRT) and fractionated stereotactic body radiation therapy (F-SBRT). For example, in Siddiqui *et al* (2009), 44 patients were treated using either single-fraction doses of 13–18 Gy or 36–48 Gy in five to eight fractions. Among the tumours analyzed in that work, a response rate (complete + PR) of 69% was seen considering only the recurrent lesions. These authors

Table 5. Results obtained in mucosa for HN patients treated in Finland (Kankaanranta *et al* 2012). All doses correspond to the maximum value administered after the first BNCT application.

		Dose			*NTCP* (⩾G3)	
Patient	^{10}B Conc. (ppm)	Absorbed (Gy)	RBE-weighted (Gy-Eq)	Photon iso-eff. (Gy(IsoE))	RBE-weighted versus photon iso-eff. dose	Observed response
#1 Mucosa (1st BNCT)	37	5.8	13.2	17.3	~0 versus 0.46	G1
#2 Mucosa (1st BNCT)	33	4.3	9.5	13.9	~0 versus ~0	G0
#3 Mucosa (1st BNCT)	36	5.9	13.2	17.4	~0 versus 0.48	G0
#4 Mucosa (1st BNCT)	38	5.8	13.2	17.3	~0 versus 0.46	G3
#5 Mucosa (1st BNCT)	34	5.6	12.2	16.4	~0 versus 0.21	G3
#6 Mucosa (1st BNCT)	43	3.9	9.0	13.8	~0 versus ~0	G2

also reported that there was no statistically significant difference between S-SBRT and F-SBRT for overall survival for all patients. Another example that involved the use of SBRT in recurrent head and neck cancer is the work of Rwigema *et al* (2015). In this article, the authors reported a CR rate of about 50% for radiation doses between 44 and 50 Gy in 5 fractions (and of around 85% if CR + PR responses are considered). If a radiation dose of 50 Gy delivered in 5 fractions is converted to a single dose, values between 21 to 25 Gy are obtained (depending on whether or not incomplete repair is included in the calculation). As mentioned above, a detailed analysis based on a statistically relevant number of clinical cases is needed to conclude that photon iso-effective doses are more adequate than RBE-weighted doses to explain the clinical outcome in BNCT in terms of photon radiation therapy. However, the closer agreement of the dose–response relationship for the photon iso-effective dose with the data reported for S-SBRT (or F-SBRT converted to single fraction) contributes to support this conclusion.

The dosimetry results obtained for the mucosa are condensed in table 5. Note that in addition to the patients analysed for tumour control with mild mucositis, this table aslo includes examples presenting more severe toxicities. As can be observed, the photon iso-effective doses in the location where the maximum occurs are higher than the corresponding RBE-weighted values. Note that the same was observed for the case of the minimum doses in the GTV, for which the values of absorbed doses are about the same as the maximum delivered absorbed doses in the mucosa (i.e. less than 6 Gy).

The normal tissue complication probabilities for acute mucosa toxicity ⩾G3, computed using the maximum doses estimated with both models of dose calculation, are compared with the observed radiation toxicities after first delivered treatment (table 5).

As in the case of tumours, the obtained results would suggest that the photon iso-effective doses are more adequate to explain the observed radiation toxicity than the RBE-weighted dose. In Kankaanranta *et al* (2012), mucosal membrane absorbed dose was limited to 6 Gy or less for each BNCT treatment carried out within the Phase I/II study in Finland (Kankaanranta *et al* 2012). The maximum value of 6 Gy corresponds to an RBE-weighted dose of less than 14 Gy-Eq which, evaluated in the dose–response curve obtained from the HN cancer radiotherapy in humans (see figure 2), gives a very low *NTCP* ($<$0.007).

This means that, if the standard model of dose calculation is correct, the probability that 3 or more patients develop toxicity ⩾G3 is less than 0.001, while the probability for 5 or more is less than $2 \cdot 10^{-6}$. After the first BNCT application, more than five treated patients developed G3. Therefore, these results show that the RBE-weighted doses are unable to predict mucosa

toxicities. On the other hand, since the photon iso-effective dose for 6 Gy of absorbed dose gives a probabilty of about 0.5, the prediction based on this model is compatible with the observed clinical outcome.

4. Conclusions

BNCT is a treatment modality that produces different LET radiations that contribute to the total dose. Since each type of radiation interacts with tissues producing a particular level of biological damage, the comparison of this technique with photon radiation therapies requires a model for dose calculations that predicts an adequate 'photon-equivalent' value.

In this work, the photon-isoffective dose formalism based on *in vivo* cancer models and human data was introduced as an alternative approach for the calculation of photon-equivalent doses in BNCT. This new model was compared with a previous version which makes use of *in vitro* cell survival data. Regardless of the nature of the experimental data used to determine the parameters of the model (i.e. *in vitro* cell survival or *in vivo* tumour control), the conclusions were consistent: photon iso-effective doses in tumour are in general lower or much lower than the ones obtained with the standard formalism. These results reinforce previous assertions that RBE-weighted tumour dose often overestimates the real photon equivalent values.

The photon iso-effective dose model was applied retrospectively to evaluate the dosimetry in tumour and dose-limiting normal tissues for head and neck patients treated with BNCT in Finland. For tumour dosimetry, although a conclusive analysis requires the inclusion of more clinical cases, the reported results suggest that the probabilities of tumour control derived from photon iso-effective doses are more adequate to explain the observed responses than those obtained with the RBE-weighted model. The same holds when dose–response BNCT data for each model are assessed in terms of the photon radiation therapy outcome.

The extension of the photon iso-effective dose model in BNCT so as to include information from dose–response assessments in animal models and humans has, for the first time, allowed the determination of the photon iso-effective dose for severe complications in the dose-limiting normal tissue. The dosimetry obtained in the mucosa for head and neck patients treated with BNCT showed that the photon iso-effective doses in the presciption point are higher than the corresponding RBE-weighted values. Based on these results, it was shown that the RBE-weighted doses are unable to predict mucosa toxicities while the prediction based on the photon iso-effective model is compatible with the observed clinical outcome.

Finally, it should be mentioned that the formalism developed in this work to compute 'photon-equivalent' doses can be of interest to other therapies that combine mixed field radiation, such as the case of hadron therapy. For this radiation treatment, charged particle mixed fields are produced after the primary beam interacts with tissue elements, giving rise to radiation components of different qualities, e.g. delta rays, primary beam particles, heavy recoils, and fragmentation products, among others. Therefore, knowing the contribution of each component and provided that radiobiological parameters for each radiation quality are available, the photon iso-effective dose model can be readily applied.

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References

Alvarez E *et al* 2003 Preclinical characterization of CG53135 (FGF-20) in radiation and concomitant chemotherapy/radiation-induced oral mucositis *Clin. Cancer Res.* **9** 3454–61

Bentzen S M, Johansen L V, Overgaard J and Thames H D 1991 Clinical radiobiology of squamous cell carcinoma of the oropharynx *Int. J. Radiat. Oncol. Biol. Phys.* **20** 1197–206

Bentzen S M *et al* 1989 Clinical radiobiology of malignant melanoma *Radiat. Oncol.* **16** 169–82

Bowen J M, Gibson R J and Keefe D M 2011 Animal models of mucositis: implications for therapy *J. Support Oncol.* **9** 161–8

Brenner D B 1992 Dose, volume and tumour-control predictions in radiotherapy *Int. J. Radiat. Oncol. Biol. Phys.* **26** 171–9

Burkart W, Jung T and Frasch G 1999 Damage pattern as a function of radiation quality and other factors *C. R. Acad. Sci.* III **322** 89–101

Chen Y K and Lin L M 2010 DMBA-induced hamster buccal pouch carcinoma and VX2-induced rabbit cancer as a model for human oral carcinogenesis *Expert Rev. Anticancer Ther.* **10** 1485–96

Coderre J A and Morris G M 1999 The radiation biology of boron neutron capture therapy *Radiat. Res.* **151** 1–18

Dagrosa M A *et al* 2007 Optimization of boron neutron capture therapy for the treatment of undifferentiated thyroid cancer *Int. J. Radiat. Oncol. Biol. Phys.* **69** 1059–66

González S J and Carando D G 2008 A general tumour control probability model for non-uniform dose distributions *Math. Med. Biol.* **2** 171–84

González S J and Santa Cruz G A 2012 The photon-isoeffective dose in boron neutron capture therapy *Radiat. Res.* **178** 609–21

González S J *et al* 2004 First BNCT treatment of a skin melanoma in Argentina: dosimetric analysis and clinical outcome *Appl. Radiat. Isot.* **61** 1101–5

Heber E M, Monti Hughes A, Pozzi E C, Itoiz M E, Aromando R F, Molinari A J, Garabalino M A, Nigg D W, Trivillin V A and Schwint A E 2010 Development of a model of tissue with potentially malignant disorders (PMD) in the hamster cheek pouch to explore the long-term potential therapeutic and/or toxic effects of different therapeutic modalities *Arch. Oral Biol.* **55** 46–51

Heber E M *et al* 2014 Therapeutic efficacy of boron neutron capture therapy mediated by boron-rich liposomes for oral cancer in the hamster cheek pouch model *Proc. Natl Acad. Sci. USA* **111** 16077–81

Hopewell J W, Morris G M, Schwint A and Coderre J A 2011 The radiobiological principles of boron neutron capture therapy: a critical review *Appl. Radiat. Isot.* **69** 1756–9

Jensen S B and Peterson D E 2014 Oral mucosal injury caused by cancer therapies: current management and new frontiers in research *J. Oral Pathol. Med.* **43** 81–90

Kankaanranta L *et al* 2012 Boron neutron capture therapy in the treatment of locally recurred head-and-neck cancer: final analysis of a phase I/II trial *Int. J. Radiat. Oncol. Biol. Phys.* **82** e67–75

Kato I, Ono K, Sakurai Y, Ohmae M, Maruhashi A, Imahori Y, Kirihata M, Nakazawa M and Yura Y 2004 Effectiveness of BNCT for recurrent head and neck malignancies *Appl. Radiat. Isot.* **61** 1069–73

Kellerer A M and Rossi H H 1972 The theory of dual radiation action *Curr. Top. Radiat. Res. Q.* **8** 85–158

Kellerer A M and Rossi H H 1978 A generalized formulation of dual radiation action *Radiat. Res.* **75** 471–88

Kreimann E L, Itoiz M E, Dagrosa A, Garavaglia R, Farías S, Batistoni D and Schwint A E 2001a The hamster cheek pouch as a model of oral cancer for boron neutron capture therapy studies: selective delivery of boron by boronophenylalanine *Cancer Res.* **61** 8775–81

Kreimann E L, Itoiz M E, Longhino J, Blaumann H, Calzetta O and Schwint A E 2001b Boron neutron capture therapy for the treatment of oral cancer in the hamster cheek pouch model *Cancer Res. Adv. Brief* **61** 8638–42

Lea D E 1946 *Action of Radiations on Living Cells* (Cambridge: Cambridge University Press)

Lea D E and Catcheside D G 1942 The mechanism of induction by radiation of chromosome aberrations in Tradescandia *J. Genet.* **44** 216–45

Locher G L 1936 Biological effects and therapeutic possibilities of neutrons *Am. J. Roentgenol.* **36** 1–13

Menéndez P R *et al* 2009 BNCT for skin melanoma in extremities: updated argentine clinical results *Appl. Radiat. Isot.* **67** 50–3
Millar W T and Hopewell J W 2007 Effects of very low dose-rate (90)Sr/(90)Y exposure on the acute moist desquamation response of pig skin *Radiother. Oncol.* **83** 187–95
Molinari A J *et al* 2011 'Sequential' boron neutron capture therapy (BNCT): a novel approach to BNCT for the treatment of oral cancer in the hamster cheek pouch model *Radiat. Res.* **175** 463–72
Molinari A J *et al* 2012 Tumour blood vessel 'normalization' improves the therapeutic efficacy of boron neutron capture therapy (BNCT) in experimental oral cancer *Radiat. Res.* **177** 59–68
Monti Hughes A *et al* 2011 Boron neutron capture therapy (BNCT) in an oral precancer model: therapeutic benefits and potential toxicity of a double application of BNCT with a six-week interval *Oral Oncol.* **47** 1017–22
Monti Hughes A *et al* 2013 Boron neutron capture therapy for oral precancer: proof of principle in an experimental animal model *Oral Dis.* **19** 789–95
Monti Hughes A, Aromando R, Pérez M A, Schwint A E and Itoiz M E 2015a The hamster cheek pouch model for field cancerization studies *Periodontology* **67** 292–311
Monti Hughes A *et al* 2015b Histamine reduces boron neutron capture therapy-induced mucositis in an oral precancer model *Oral Dis.* **21** 770–7
Monti Hughes A *et al* 2016 Topical application of histamine gel would protect oral precancerous tissue from BNCT induced mucositis but would affect therapeutic effect on tumours: preliminary studies in an oral cancer model *Proc. of the 17th ICNCT (Columbia MO, USA)*
Nagini S, Letchoumya P V, Thangavelu A and Ramachandran C 2009 Of humans and hamsters: a comparative evaluation of carcinogen activation, DNA damage, cell proliferation, apoptosis, invasion, and angiogenesis in oral cancer patients and hamster buccal pouch carcinomas *Oral Oncol.* **45** e31–7
Pozzi E *et al* 2009 Dosimetry and radiobiology at the new RA-3 reactor boron neutron capture therapy (BNCT) facility: application to the treatment of experimental oral cancer *Appl. Radiat. Isot.* **67** S309–12
Pozzi E C *et al* 2012 Boron neutron capture therapy (BNCT) for liver metastasis: therapeutic efficacy in an experimental model *Radiat. Environ. Biophys.* **51** 331–9
Pozzi E C *et al* 2013 Boron neutron capture therapy (BNCT) for liver metastasis in an experimental model: dose–response at five-week follow-up based on retrospective dose assessment in individual rats *Radiat. Environ. Biophys.* **52** 481–91
Provenzano L *et al* BNCT treatments of head and neck cancer at the RA-6 reactor: from preclinical studies in dogs to feasibility assessments in humans submitted
Rwigema J C *et al* 2015 4π noncoplanar stereotactic body radiation therapy for head-and-neck cancer: potential to improve tumour control and late toxicity *Int. J. Radiat. Oncol. Biol. Phys.* **91** 401–9
Schmid T E *et al* 2010 Differences in the kinetics of γ-H2AX fluorescence decay after exposure to low and high LET radiation *Int. J. Radiat. Biol.* **86** 682–91
Siddiqui F, Patel M, Khan M, McLean S, Dragovic J, Jin J Y, Movsas B and Ryu S 2009 Stereotactic body radiation therapy for primary, recurrent, and metastatic tumours in the head-and-neck region *Int. J. Radiat. Oncol. Biol. Phys.* **74** 1047–53
Strigari L, Pedicini P, D'Andrea M, Pinnarò P, Marucci L, Giordano C and Benassi M 2012 A new model for predicting acute mucosal toxicity in head-and-neck cancer patients undergoing radiotherapy with altered schedules *Int. J. Radiat. Oncol. Biol. Phys.* **83** e697–702
Stüben G, van der Kogel A J and van der Schueren E 1997 Biological equivalence of low dose rate to multifractionated high dose rate irradiations: investigations in mouse lip mucosa *Radiother. Oncol.* **42** 189–96
Supsavhad W, Dirksen W P, Martin C K and Rosol T J 2016 Animal models of head and neck squamous cell carcinoma *Vet. J.* **210** 7–16
Suzuki S 1998 A theoretical model for simultaneous mixed irradiation with multiple types of radiation *J. Radiat. Res.* **39** 215–21
Thames H D, Bentzen S M, Turesson I, Overgaard M and van den Bogaert W 1989 Fractionation parameters for human tissues and tumours *Int. J. Radiat. Biol.* **56** 701–10
Trivillin V A, Heber E M, Nigg D W, Itoiz M E, Calzetta O, Blaumann H, Longhino J and Schwint A E 2006 Therapeutic success of boron neutron capture therapy (BNCT) mediated by a chemically non-selective boron agent in an experimental model of oral cancer: a new paradigm in BNCT radiobiology *Radiat. Res.* **166** 387–96

Vairaktaris E, Spyridonidou S, Papakosta V, Vylliotis A, Lazaris A, Perrea D, Yapijakis C and Patsouris E 2008 The hamster model of sequential oral oncogenesis *Oral Oncol.* **44** 315–24

Wang L W *et al* 2014 Fractionated BNCT for locally recurrent head and neck cancer: experience from a phase I/II clinical trial at Tsing Hua open-pool reactor *Appl. Radiat. Isot.* **88** 23–7

Wu X, Chen P, Sonis S T, Lingen M W, Berger A and Toback F G 2012 A novel peptide to treat oral mucositis blocks endothelial and epithelial cell apoptosis *Int. J. Radiat. Oncol. Biol. Phys.* **83** e409–15

Zaider M and Rossi H H 1980 The synergistic effects of different radiations *Radiat. Res.* **83** 732–9