The Canadian Neutron-Induced Carcinogenic Effects Research Program

A research program to investigate neutron relative biological effectiveness for carcinogenesis with a particular focus on secondary (by-product) neutrons in high-energy radiation therapy

John Kildea[1,2]

[1] Medical Physics Unit, Department of Oncology, McGill University, DS1.7141, Cedars Cancer Centre, 1001 boul Décarie, Montréal, Québec, H4A 3J1, Canada

[2] Cancer Research Program, McGill University Health Centre Research Institute, 1001 boul Décarie, Montréal, Québec, H4A 3J1, Canada

*Corresponding author: John Kildea, Medical Physics Unit, Department of Oncology, McGill University, DS1.7141, Cedars Cancer Centre, 1001 boul Décarie, Montréal, Québec, H4A 3J1, Canada

TEL & FAX: +1 514 934 1934 ex 44154

E-mail address: john.kildea@mcgill.ca (Dr. John Kildea)

Conflict of Interest statement; The author reports no conflicts of interest.

Abstract:

Industrial exposure to neutron irradiation is tightly controlled such that the carcinogenic risk to individuals and populations is very low. Patients undergoing high-energy (>10 MeV) radiation therapy, however, represent a population group for whom whole-body neutron exposure cannot be controlled. Such patients are thus at risk for second radiation-induced malignancies. This is true in particular for cured pediatric cancer patients whose life expectancy exceeds the latency period for radiation-induced tumor formation. The Neutron-Induced Carcinogenic Effects (NICE) research program (depdocs.com/jkildea/NICE.html) is designed to study the biophysics underlying the energy-dependent variation in the carcinogenic potential of neutrons such that the second cancer risk to radiation therapy patients may be better understood and accounted for. The program calls for macroscopic and microscopic Monte Carlo modelling of real-world polyenergetic and non-isotropic neutron sources under experimental irradiation conditions coupled with radiobiological experiments and DNA-damage assays. The five-year research program is a collaboration between McGill University, Canadian Nuclear Laboratories, the Canadian Nuclear Safety Commission and Detec Inc.

1. Introduction

Neutron radiation is encountered in space, during high-altitude air travel, at nuclear power plants, in various industrial applications, and in radiation therapy (RT). As is the case for all ionizing radiations, neutrons pose a risk to human health that must be mitigated. Current radiological protection measures [1], as codified in laws and regulations, are designed to protect human populations from the biological risk that neutrons (and other forms of radiation) pose. While these measures are generally adequate, they do not—and cannot—protect patients undergoing high-energy (>10 MeV) photon or proton RT (HEPP-RT). Neutrons that are generated as secondary, by-product, radiation during these RT treatments, cannot be shielded and consequently exposed patients are susceptible to radiation-carcinogenesis [2, 3, 4, 5]. The carcinogenic risk from HEPP-RT, a few percent (eg [3] estimated an absolute 1.4% risk, or 14 patients per 1000, for 10-year survivors of prostate cancer), is well known and generally accepted, although poorly understood [6]. Recent efforts to expand the use of proton beam RT worldwide [7, 8, 9] have brought the issue into focus [10]. Paediatric patients, who are generally considered the main beneficiaries of proton RT [8], are the most at risk for iatrogenic second malignancies resulting from the unavoidable whole body dose of secondary radiation that arises in part from neutrons [5, 11].

Our Neutron-Induced Carcinogenic Effects (NICE) research program (depdocs.com/jkildea/NICE.html) is motivated by the need to better understand the biophysics of dose deposition by secondary neutrons in HEPP-RT so that their

biological effects can be mitigated during RT planning. The impact of our research, however, is independent of HEPP-RT and extends to all situations where neutron radiation is encountered.

1.2 Current Knowledge

Ionizing radiation has, by definition, the potential to alter the material through which it passes—it may ionize the atoms and molecules it encounters. While this is true for all materials, ionizing radiation has the particular ability to inflict damage to the DNA found in the cells of living tissue [12]. DNA damage is beneficial in the context of killing targeted cancer cells in RT but it otherwise presents a risk to healthy tissue, which may experience immediate cell death or long-term mutation-induced carcinogenesis [13, 14]. Neutrons are a particularly insidious form of ionizing radiation, especially from the standpoint of carcinogenesis. On one hand, they readily scatter off all material that they encounter, rendering them difficult to control. On the other hand, due to the complex energy-dependent spectra of secondary particles that are produced by neutron interactions, their carcinogenic potential is energy dependent, necessitating knowledge of the neutron energy spectrum (a difficult task) in order to quantify the risk from any given exposure situation [15].

Our current knowledge of neutron carcinogenic potential is encapsulated in the highly uncertain radiation weighting factors (W_Rs) for neutrons promulgated by the International Commission on Radiological Protection (ICRP) [1]. As shown in Figure 1,

these weighting factors, which are used to convert absorbed dose (in gray) into a more biologically-meaningful equivalent dose (in sievert), vary as a function of energy, peaking at a value of 20 for neutrons around 1 MeV. For photons and electrons, by contrast, the radiation weighting factors are unity for all energies. The ICRP never intended the W_Rs to be used for individual risk predictions [16] and as such, they offer little value in the context of individual patient-physician decision-making in radiation therapy treatment planning.

Recent work by the European ANDANTE collaboration [6, 17, 18] has provided important credence to the ICRP's W_Rs for neutrons. Using first-principles transport of secondary particles arising from isotropic monoenergetic primary neutrons and a track-structure analysis of DNA damage weighted by relative secondary particle population, the ANDANTE team were able to broadly reproduce the shape of the ICRP's energy-dependent variation of W_Rs for neutrons [19, 20]. Although radiobiological experiments by the ANDANTE team did not reveal any biological evidence for variation in carcinogenic potential as a function of energy [18], their modelling results provide sufficient impetus to extend their approach of coupling transport and track-structure simulations to real-world polyenergetic scenarios where radiobiological evidence may be more forthcoming.

1.3 Recent progress in neutron spectral measurements

Neutron spectral measurements around photon/electron RT linear accelerators and

proton-therapy cyclotrons have traditionally been difficult and time-consuming undertakings. The high dose-rate encountered in the HEPP-RT environment has prohibited active readout techniques due to pulse pile-up, while passive techniques have required long irradiation times and unwieldy post-irradiation readouts. Recently, however, our group has shown that a new type of neutron spectrometer, the Nested Neutron Spectrometer (NNS) [21] may be used to reliably measure the neutron spectrum for a single position in an RT treatment room in less than one hour, including setup and data unfolding [22, 23]. When operated in an active current-mode readout (akin to the work of [24]), the NNS avoids the issue of pulse pile-up and facilitates much shorter irradiation times. Furthermore, the practical nested cylindrical design of the NNS moderators allows for a reduction in setup time compared to traditional Bonner spheres. The NNS thus represents a new, fast, and practical method for neutron spectral measurements in HEPP-RT.

1.4 A Word about Neutron Therapy and Clinical Decision-making

Any discussion involving neutron-induced carcinogenesis and medicine naturally evokes questions regarding clinical decision-making in the context of neutrons. Why not just avoid neutrons completely if they pose a carcinogenic risk for the patient? The choice in front of the patient, however, is never so simple and it necessarily involves an informed discussion between the clinician and the patient.

In some cases, neutron therapy itself, either fast neutron therapy (FNT) or boron

neutron capture therapy (BNCT), may be warranted and the potential short-term benefits to the patient may (far) outweigh the long-term carcinogenic risk. Indeed, clinical cases for which neutron beam therapy are indicated typically involve radio-resistant or hypoxic tumors or second-line rescue/salvage treatments with otherwise poor prognosis (see for example [25, 26] regarding FNT and [27-31] regarding BNCT). The issue of a neutron-induced cancer in five, ten or twenty years is essentially irrelevant in such circumstances. Furthermore, in the case of BNCT, the neutron energies involved (thermal or epithermal) allow for shielding such that the whole-body neutron dose is extremely low.

In HEPP-RT, it is secondary (by-product) neutrons that are at play. Again, individual clinical circumstances dictate whether the (low) long-term carcinogenic risk is outweighed by the therapeutic gain. For older patients and palliative treatments, carcinogenic concerns are secondary and the immediate therapeutic effect of the photon or proton beams is paramount. The situation is different, however, for pediatric patients who, if cured of their immediate malignancy, may live long enough such that the risk of a second radiation-induced cancer is significant [2]. It is thus for the particular benefit of pediatric patients that we should attempt to improve our understanding of neutron-induced carcinogenesis.

2. Overview of the NICE Research Program

The long-term goal of our NICE research program is focused on improving

second-malignancy risk estimates for patients undergoing HEPP-RT. The five-year program (2016-2021) is a collaboration between researchers at McGill University, Canadian Nuclear Laboratories (Chalk River, Ontario), the Canadian Nuclear Safety Commission and Detec Inc.. Funding is provided by the Natural Sciences and Engineering Research Council of Canada (Discovery Grants program, PI: Kildea) and Canadian Nuclear Laboratories, with in-kind support provided by all collaborating organisations.

2.1 Research Program Rationale

Cancer risk estimates for HEPP-RT must ultimately account for both out-of-field photon and neutron irradiation. Although the out-of-field neutron absorbed dose in HEPP-RT amounts to just about one quarter of the photon component, the NICE research program will initially focus on neutrons. The motivation for this focus is twofold. (i) The carcinogenic potential of neutrons is high compared to photons. As already stated, the presently-accepted ICRP radiation weighting factors for neutrons reach twenty times the value of their photon counterparts at 1 MeV. This, unfortunately, is also the energy at which most secondary neutrons are produced in HEPP-RT by the dominant nuclear evaporation process [32]. (ii) The anticipated energy-dependent carcinogenic potential of neutrons provides us with a unique smoking-gun opportunity to predict and experimentally examine DNA damage for distinct neutron spectra for which the carcinogenic potential is expected to be very different. The potential to better

understand the underlying biophysical cause of radiation-carcinogenesis using neutrons is thus significant.

As always, and as is the case in neutron therapy, the decision to treat with neutron-producing photon and proton RT beams will remain a clinical one. As stated, the present research is attempting to inform our understanding of the carcinogenic risk from neutrons. However, absolute quantification of that risk, particularly for pediatric patients, require long-term outcomes data coupled with accurate neutron dosimetry.

2.2 Research Program Objectives

To examine the biophysics underlying neutron DNA damage, the NICE research program has identified the following five objectives (please see Figure 2 for a visual summary):

1. *Characterization of neutron sources:* Fully characterize the spectra of the various neutron sources available to the program (photon/electron RT linacs at the McGill University Health Centre, neutron generators and reactor neutron beams at CNL, and future proton therapy partners) using a Nested Neutron Spectrometer and Monte Carlo modelling.

2. *Neutron spectra under realistic experimental conditions:* Computationally transport neutrons from the aforementioned sources to points of interest under various experimental exposure conditions (e.g. positions in an anthropomorphic phantom and positions in a cell culture medium) using Monte Carlo modelling,

validated by measurements with a Nested Neutron Spectrometer.

3. *Selection of experimental conditions with most distinctive carcinogenic potentials:* Select the experimental conditions that should (according to the currently-accepted ICRP radiation weighting factors) provide distinctly different carcinogenic potentials (e.g. source spectra with very different thermal and fast neutron components and/or points of interest that experience very different secondary particle populations).

4. *Track structure simulations of DNA damage:* For the selected experimental conditions, score DNA damage at the points of interest using track structure simulations. Similarly, examine DNA damage by ionizing photons as a control.

5. *Examine the carcinogenic potential of each neutron source*: Irradiate human lymphocyte cells (with neutrons and photons) under the selected experimental conditions and measure the corresponding DNA damage using various biodosimetry assays.

3. Action Plans

The NICE research program incorporates a specific action plan for each of the five objectives listed above.

3.1 Characterization of neutron sources

This initial action plan will characterize the in-air strength, energy spectra, and photon

contamination of each of the neutron sources available to the research program. We have immediate access to two dedicated neutron sources at CNL; one for thermalized neutrons from the National Research Universal reactor (A in Figures 1, 2), the other for ~2.7 MeV neutrons from a Deuterium-Deuterium generator at the Health Physics Neutron Generator (B in Figures 1, 2), and to photoneutrons produced by clinical RT photon beams at the McGill University Health Centre (C in Figures 1, 2). Access to specific clinical proton therapy beams has yet to be finalized. Characterisation of the neutron sources will entail Monte Carlo modelling of each neutron source, including full room scatter, validated with in-air spectral measurements using a Nested Neutron Spectrometer. These measurements are akin to our previously-published work [23]. For our Monte Carlo modelling we plan to use the Geant4 package [33] due to previous experience with it.

3.2 Neutron spectra under realistic experimental conditions

In this second action plan, we will use our measurement-validated Monte Carlo source spectra as starting conditions from which we will model neutron spectra at depth in an anthropomorphic phantom and for cells within a culture medium. Respectively, these represent realistic irradiation conditions for HEPP-RT patients and for radiobiology experiments at CNL. The reason for this effort is the need to fully understand the secondary charged particle spectra under the conditions in which we will subsequently examine neutron dose deposition and DNA damage. Since neutrons readily scatter and

are moderated by all material that they encounter, and since nuclear reaction cross sections depend on energy, it is necessary to know not only the in-air room-moderated spectrum for each (polyenergetic and non-isotropic) neutron source but also the unique particle-energy spectrum at the ultimate point of measurement. It is the particle-energy spectrum of the secondary particles that ultimately translates into the biologically-destructive absorption of ionizing radiation.

3.3 Selection of experimental conditions with most distinctive carcinogenic potentials

Available evidence suggests that the carcinogenic potential of neutrons peaks at 1 MeV and falls off at either side of this value (Figure 1, [1, 19]). Under ideal conditions, which are available in Monte Carlo, we would compare DNA damage by monoenergetic neutron beams at thermal energies, at 1 MeV, and at 10 MeV or above. The ANDANTE studies reported by Baiocco et al., [18,19] employed such an approach and demonstrated that it is a very worthwhile exercise. In reality, however, we are constrained to work experimentally with broad, scatter-moderated, neutron beams. That said, considering the range of neutron sources available to us (see Figure 1), it will be possible to select two (or possibly more) irradiation conditions for which the neutron carcinogenic potentials are expected to be most different. These will be selected by considering the existing ICRP weighting factors coupled with knowledge of the charged particle populations at the points of interest. The selected experimental conditions will then form the starting points for track structure simulations and for radiobiological

measurements at CNL.

3.4 Track structure simulations of DNA damage

In this action plan we will carry out a three-step study of neutron dose deposition in human cells using Monte Carlo track structure methods. The study will be repeated for each of our two (or more), previously-determined, experimental neutron irradiation conditions that show greatest difference in carcinogenic potential. For control, and for the calculation of values of Relative Biological Effectiveness (RBE) the study will also be repeated for ionizing photons.

Step 1: At the macroscopic scale, score the frequency, spatial, and energy distributions of the secondary charged particles and electrons that make it to our points of interest (in phantom and/or in cell culture).

Step 2: For each secondary particle type identified in step 1, we will quantify at the nanoscopic scale its ability to produce single strand breaks (SSBs), double strand breaks (DSBs), and clustered lesions across both strands of the DNA macromolecule. These endpoints will provide us with surrogate indicators for neutron-induced carcinogenic potential.

Step 3: We will combine the carcinogenic potential of each particle type from step 2 with its corresponding relative importance in the secondary particle population established in step 1. This will provide us with a resultant carcinogenic potential for the neutron irradiation conditions in question.

The rationale for this three-step multiscale approach is based on current understanding of radiation damage to DNA, involving SSBs, DSBs, and clusters [12], as well as on the knowledge that neutron interactions may produce a plethora of secondary charged particle types. It is similar to the methodology employed by the European BioQuaRT project (Biologically weighted Quantities in RadioTherapy), [34] which is combining track structure modelling and radiobiological measurements to study the effects of protons and light ions in RT. It is also similar to the work of the ANDANTE collaboration [6, 17, 18] in so far as it couples macroscopic radiation transport and microscopic track structure modelling. However, rather than following the *ab-initio* approach of ANDANTE, with isotropic and monoenergetic starting conditions, the NICE project will begin with realistic experimental conditions and then select out for further analysis those conditions that are most suggestive of distinct carcinogenic potentials.

Several Monte Carlo codes are available for the purpose of nanoscale track structure modelling with full DNA and cellular macromolecule representation. These include the Monte Carlo Damage Simulation (MCDS) [35], PARTRAC [36], and Geant4-DNA [37] packages. For this research we plan to initially use MCDS, as it is the track structure code with which we have the most experience. However, as we plan to use Geant4 for our macroscale studies, we envisage an eventual transition to Geant4-DNA. This will allow us to study macroscale neutron spectra and nanoscale DNA damage using a single Monte Carlo framework. Neutron transport is not yet

supported in Geant4-DNA and some development and validation will be required.

3.5 Examine the carcinogenic potential of each neutron source

Results from our Monte Carlo track structure studies (i.e. scoring of SSBs, DSBs and clusters as indicators for carcinogenesis) will ultimately be compared against radiobiological measurements to be carried out at CNL's Biological Research Facility. The CNL team will expose human lymphocyte cells to neutrons *in vitro*. The experimental conditions to be used at CNL will match those used in our track structure study. CNL operate a biodosimetry laboratory as part of the Canadian biodosimetry network [38] and have the facilities and expertise to conduct automated and manual chromosomal damage assays. A number of assays are under consideration, including the dicentric chromosome assay (DCA) [39], the γ-H2AX assay [40], fluorescence in situ hybridization (FISH) [41] and micronuclei assays [42]. All are available at CNL. DCA is the gold-standard assay for biodosimetry [39] and, as such, it is our initial and primary choice for the radiobiological measurements of the NICE program. Results from the CNL study will validate/refute/inform the results of our proposed Monte Carlo and track structure study.
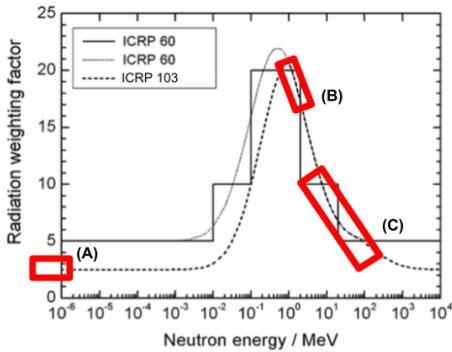
4. Impact

This research revolves around examining the biophysics at play in the induction of DNA damage by neutrons. Areas of significance include accurate Monte Carlo

modelling of realistic neutron irradiation conditions validated by measurements with a new type of practical neutron spectrometer that may be reliably used in the HEPP-RT environment, and novel track structure-based metrics for carcinogenic potential tested against experimental radiobiological data. The results of this research will be of interest to the radiation oncology and medical physics communities in light of recent results of the ANDANTE collaboration and in the context of the continued use of high-energy photon and proton beams in RT.

Figure 1: Radiation weighting factors for neutrons as a function of energy as recommended by the ICRP [1]. Approximate energies of the neutron sources available to the NICE project are represented in red. (A) thermal neutrons at CNL, (B) ~2.7 MeV Deuterium-Deuterium neutrons at CNL, and (C) neutrons available in RT (photon or proton therapy facilities).
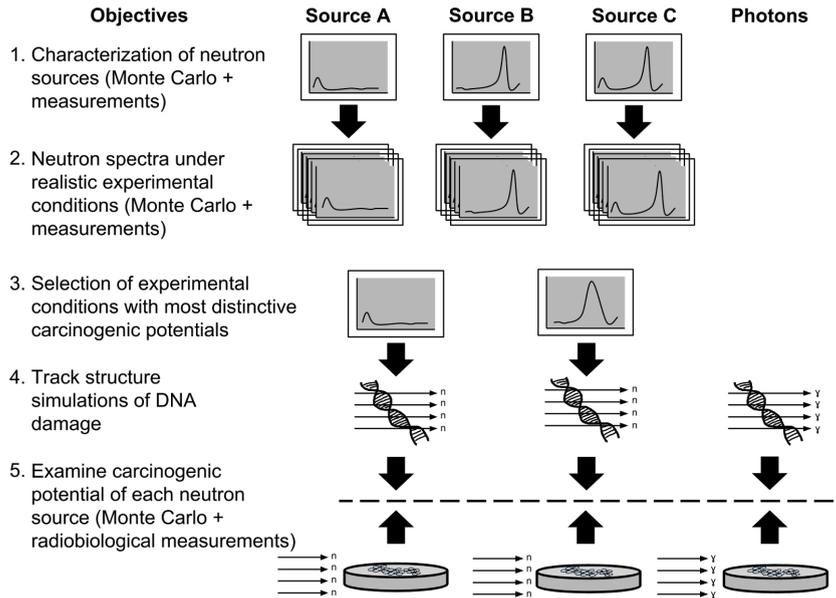
Figure 2: Proposed objectives for our experimental and Monte Carlo studies of the biophysics underlying neutron-induced carcinogenesis.

References

1. The 2007 recommendations of the International Commission on Radiological Protection. Oxford: Elsevier, 2007.

2. Hall, E. J. (2006). Intensity-modulated radiation therapy, protons, and the risk of second cancers. International Journal of Radiation Oncology Biology Physics, 65(1), 1-7.

3. Brenner, D. J., Curtis, R. E., Hall, E. J., & Ron, E. (2000). Second malignancies in prostate carcinoma patients after radiotherapy compared with surgery. Cancer, 88(2), 398-406.

4. Kry, S. F., Salehpour, M., Followill, D. S., Stovall, M., Kuban, D. A., White, R. A., & Rosen, I. I. (2005). The calculated risk of fatal secondary malignancies from intensity-modulated radiation therapy. International Journal of Radiation Oncology Biology Physics,62(4), 1195-1203.

5. Tukenova, M., Guibout, C., Hawkins, M., Quiniou, E., Mousannif, A., Pacquement, H., ... & de Vathaire, F. (2011). Radiation therapy and late mortality from second sarcoma, carcinoma, and hematological malignancies after a solid cancer in childhood. International Journal of Radiation Oncology Biology Physics, 80(2), 339-346.

6. Ottolenghi, A., Smyth, V., & Trott, K. R. (2013). Assessment of cancer risk from neutron exposure–The ANDANTE project. Radiation Measurements, 57, 68-73.

7. Newhauser, W. D., & Zhang, R. (2015). The physics of proton therapy. Physics in medicine and biology, 60(8), R155.

8. Mohan, R., & Grosshans, D. (2016). Proton therapy–Present and future. Advanced Drug Delivery Reviews.

9. Toltz, A., Shin, N., Mitrou, E., Laude, C., Freeman, C. R., Seuntjens, J., ... & Roberge, D. (2015). Late radiation toxicity in Hodgkin lymphoma patients: proton therapy's potential. Journal of Applied Clinical Medical Physics, 16(5).

10. Brenner, D. J., & Hall, E. J. (2008). Secondary neutrons in clinical proton radiotherapy: a charged issue. Radiotherapy and Oncology, 86(2), 165-170.

11. Diallo, I., Haddy, N., Adjadj, E., Samand, A., Quiniou, E., Chavaudra, J., ... & de Vathaire, F. (2009). Frequency distribution of second solid cancer locations in relation to the irradiated volume among 115 patients treated for childhood cancer. International Journal of Radiation Oncology Biology Physics, 74(3), 876-883.

12. Hall, E. J., & Giaccia, A. J. (2006). Radiobiology for the Radiologist. Lippincott Williams & Wilkins.

13. Hada, M., & Georgakilas, A. G. (2008). Formation of clustered DNA damage after high-LET irradiation: a review. Journal of radiation research, 49(3), 203-210.

14. Coleman, C. N., Blakely, W. F., Fike, J. R., MacVittie, T. J., Metting, N. F., Mitchell, J. B., ... & Wong, R. S. (2003). Molecular and Cellular Biology of

Moderate-Dose (1-10 Gy) Radiation and Potential Mechanisms of Radiation Protection: Report of a Workshop at Bethesda, Maryland, December 17-18, 2001 1. Radiation research, 159(6), 812-834.

15. Followill, D. S., Stovall, M. S., Kry, S. F., & Ibbott, G. S. (2003). Neutron source strength measurements for Varian, Siemens, Elekta, and General Electric linear accelerators. Journal of applied clinical medical physics, 4(3), 189-194.

16. Valentin, J. (2003). Relative biological effectiveness (RBE), quality factor (Q), and radiation weighting factor (w R): ICRP Publication 92. Annals of the ICRP, 33(4), 1-121.

17. Ottolenghi, A., Baiocco, G., Smyth, V., & Trott, K. (2015). The ANDANTE project: a multidisciplinary approach to neutron RBE. Radiation protection dosimetry, 166(1-4), 311-315.

18. European Commission : CORDIS : Projects & Results Service : Multidisciplinary evaluation of the cancer risk from neutrons relative to photons using stem cells and the induction of second malignant neoplasms following paediatric radiation therapy http://cordis.europa.eu/project/rcn/100540_en.html

19. Baiocco, G., Alloni, D., Babini, G., Mariotti, L., & Ottolenghi, A. (2015). Reaction mechanism interplay in determining the biological effectiveness of neutrons as a function of energy. Radiation protection dosimetry, ncv134.

20. Baiocco, G., Barbieri, S., Babini, G., Morini, J., Alloni, D., Friedland, W., ... & Ottolenghi, A. (2016). The origin of neutron biological effectiveness as a

function of energy. Scientific Reports, 6.

21. Dubeau, J., Witharana, S. H., Atanackovic, J., Yonkeu, A., & Archambault, J. P. (2012). A neutron spectrometer using nested moderators. Radiation protection dosimetry, 150(2), 217-222.

22. Maglieri, R. (2015) A study of photoneutron spectra around high-energy medical linear accelerators using Monte Carlo simulations and measurements, McGill University M.Sc. thesis

23. Maglieri, R., Licea, A., Evans, M., Seuntjens, J., & Kildea, J. (2015). Measuring neutron spectra in radiotherapy using the nested neutron spectrometer. Medical Physics, 42(11), 6162-6169.

24. Hagiwara, M., Sanami, T., Iwamoto, Y., Arakawa, H., Shigyo, N., Mokhov, N., ... & Oishi, K. (2011). Shielding experiments at high energy accelerators of Fermilab (III): neutron spectrum measurements in intense pulsed neutron fields of the 120-GeV proton facility using a current bonner sphere technique. pulse, 5, 9.

25. Davis, C., Sikes, J., Namaranian, P., Laramore, G., & Dillon, J. K. (2016). Neutron beam radiation therapy: an overview of treatment and oral complications when treating salivary gland malignances. Journal of Oral and Maxillofacial Surgery, 74(4), 830-835.

26. Chapman, T. R., Laramore, G. E., Bowen, S. R., & Orio, P. F. (2016). Neutron radiation therapy for advanced thyroid cancers. Advances in Radiation

Oncology, 1(3), 148-156.

27. Haapaniemi, A., Kankaanranta, L., Saat, R., Koivunoro, H., Saarilahti, K., Mäkitie, A., ... & Joensuu, H. (2016). Boron neutron capture therapy in the treatment of recurrent laryngeal cancer. International Journal of Radiation Oncology* Biology* Physics, 95(1), 404-410.

28. Wang, L. W., Chen, Y. W., Ho, C. Y., Liu, Y. W. H., Chou, F. I., Liu, Y. H., ... & Liu, C. S. (2016). Fractionated boron neutron capture therapy in locally recurrent head and neck cancer: a prospective phase I/II trial. International Journal of Radiation Oncology* Biology* Physics, 95(1), 396-403.

29. Fuwa, N., Suzuki, M., Sakurai, Y., Nagata, K., Kinashi, Y., Masunaga, S., ... & Nakamura, T. (2014). Treatment results of boron neutron capture therapy using intra-arterial administration of boron compounds for recurrent head and neck cancer. The British journal of radiology.

30. Kawabata, S., Miyatake, S. I., Nonoguchi, N., Hiramatsu, R., Iida, K., Miyata, S., ... & Michiue, H. (2009). Survival benefit from boron neutron capture therapy for the newly diagnosed glioblastoma patients. Applied Radiation and Isotopes, 67(7), S15-S18.

31. Yamamoto, T., Nakai, K., Kageji, T., Kumada, H., Endo, K., Matsuda, M., ... & Matsumura, A. (2009). Boron neutron capture therapy for newly diagnosed glioblastoma. Radiotherapy and oncology, 91(1), 80-84.

32. Barquero, R., Edwards, T. M., Iniguez, M. P., & Vega-Carrillo, H. R. (2005).

Monte Carlo simulation estimates of neutron doses to critical organs of a patient undergoing 18MV x-ray LINAC-based radiotherapy. Medical physics, 32(12), 3579-3588.

33. Agostinelli, S., Allison, J., Amako, K. A., Apostolakis, J., Araujo, H., Arce, P., ... & Howard, A. (2003). GEANT4—a simulation toolkit. Nuclear instruments and methods in physics research section A: Accelerators, Spectrometers, Detectors and Associated Equipment, 506(3), 250-303.

34. Patrono, C., Gil, O. M., Giesen, U., Langner, F., Pinto, M., Rabus, H., & Testa, A. (2015). 'BioQuart Project: Design of a novel in situ protocol for the simultaneous visualisation of chromosomal aberrations and micronuclei after irradiation at microbeam facilities'. Radiation protection dosimetry, ncv160.

35. Semenenko, V. A., & Stewart, R. D. (2006). Fast Monte Carlo simulation of DNA damage formed by electrons and light ions. Physics in medicine and biology, 51(7), 1693.

36. Friedland, W., Dingfelder, M., Kundrát, P., & Jacob, P. (2011). Track structures, DNA targets and radiation effects in the biophysical Monte Carlo simulation code PARTRAC. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 711(1), 28-40.

37. Incerti, S., Baldacchino, G., Bernal, M., Capra, R., Champion, C., Francis, Z., ... & Zacharatou, C. (2010). The Geant4-DNA project. International Journal of Modeling, Simulation, and Scientific Computing, 1(02), 157-178.

38. Wilkins, R. C., Beaton-Green, L. A., Lachapelle, S., Kutzner, B. C., Ferrarotto, C., Chauhan, V., ... & Flegal, F. N. (2015). Evaluation of the annual Canadian biodosimetry network intercomparisons. International journal of radiation biology, 91(5), 443-451.

39. Flegal, F. N., Devantier, Y., Marro, L., & Wilkins, R. C. (2012). Validation of QuickScan dicentric chromosome analysis for high throughput radiation biological dosimetry. Health physics, 102(2), 143-153.

40. Rothkamm, K., & Horn, S. (2009). gamma-H2AX as protein biomarker for radiation exposure. Ann Ist Super Sanita, 45(3), 265-71.

41. Price, C. M. (1993). Fluorescence in situ hybridization. Blood reviews, 7(2), 127-134.

42. Aardema, M. J., & Kirsch-Volders, M. (2001). The in vitro micronucleus assay. Genetic Toxicology and Cancer Risk Assessment, 163-186.