IL NUOVO CIMENTO Vol. 34 C, N. 1 Gennaio-Febbraio 2011
DOI 10.1393/ncc/i2011-10818-9

Colloquia: IFA 2010

Boron Neutron Capture Therapy research in Pavia, Italy

S. Bortolussi

INFN, Sezione di Pavia and Dipartimento di Fisica Nucleare e Teorica, Università di Pavia via Bassi 6, 27100 Pavia, Italy

(ricevuto il 6 Luglio 2010; approvato il 25 Agosto 2010; pubblicato online l'1 Marzo 2011)

Summary. — Boron Neutron Capture Therapy is an experimental form of radiotherapy based on the enrichment of tumor cells with ^{10}B and on the subsequent irradiation with thermal neutrons. The neutron capture reaction in ^{10}B gives rise to two high-LET particles which can deliver a lethal dose to the tumor, substantially sparing the normal tissues. The selectivity of the therapy is due to the capacity of certain drugs to vehiculate the boron inside the tumor more than in the normal cells. This characteristic makes BNCT an option for tumors that have presently no cure, because surgery, conventional radiotherapy or chemotherapy are not applicable or are not much effective. One of these cases is represented by diffuse tumors, such as metastatic spreads invading entire organs. At the University of Pavia, a long research has been carried out to apply BNCT to different kinds of diffuse tumors, such as liver metastases. Two patients affected by this pathology were treated at the TRIGA reactor of the University. This paper describes the state-of-art of BNCT research in Pavia and the recent project for BNCT of osteosarcoma.

PACS 87.53.Jw – Therapeutic applications, including brachytherapy.
PACS 87.53.Bn – Dosimetry/exposure assessment.
PACS 87.55.dh – Tissue response.
PACS 87.56.jk – Field shaping.

1. – Introduction

Boron Neutron Capture Therapy (BNCT) [1] is an experimental radiotherapy based on a two-step procedure: infusion of the patient with a ^{10}B carrier and irradiation of the tumor target with thermal neutrons. The carrier must be able to concentrate boron in tumor cells more than in normal ones. The thermal neutron capture in ^{10}B takes place with a cross section of 3834 b, and the two high LET particles rising from the reaction $^{10}\mathrm{B}, (\mathrm{n}, \alpha)^{7}\mathrm{Li}$, have a range in tissue comparable to a typical cell diameter. This way, the tumor cells absorb a lethal radiation dose, and the high-LET particles which are produced inside them, do not cause damage to the surrounding cells. BNCT selectivity is not due to the radiation field, as in conventional radiotherapy, but rather to boron biodistribution obtained with the used carrier. If the drug is effective in concentrating boron

in tumor more than in normal tissue, the irradiation will target the tumor wherever it is located in the target, even if it is disseminated within the normal tissue. This situation is typical in case of metastatic spreads or when the tumor is infiltrative. In these cases, surgery is difficult and often not resolutive.

BNCT is currently applied to different kinds of tumors, such as head and neck recurrent cancer, brain tumors, skin melanoma. The countries in which clinical trials are being carried on are: Finland [2], Japan [3] and Argentina [4]. There are many research projects all over the world to apply BNCT to lung [5], oral [6], liver [7] and bone [8] tumors.

BNCT research started in Pavia([1]) during the eighties, with the aim to set-up a treatment able to treat all the metastases from colon carcinoma invading the liver. In fact, the primary tumor affecting the colon gives rise to hepatic metastases in a large percentage of cases, and almost always the patient has a poor prognosis because the organ is completely invaded and not operable. As the metastases are multifocal and often they are too small to be detected, the possibility to irradiate the entire organ, after the tumor enrichment with ^{10}B, could be a good solution. To this purpose, an innovative technique based on the organ explantation, followed by irradiation and re-implantation in the patients was conceived. Although this method presents many advantages, it is clear that the patients have to undergo a very invasive surgery. Another class of tumors that could take advantage from BNCT are lung tumors. Many primary tumors and metastastic spreads invading lungs are not curable with the available techniques, moreover standard radiotherapy is limited by the positions of the lungs, close to very radiosensitive organs. In this case, BNCT can be conceived as an external radiotherapy, because the lung tissue has a low density compared to other soft tissues, and it is possible to obtain a uniform thermal neutron field inside the lung irradiating with multiple epithermal external neutron beams. The research in this field started in Pavia some years ago and the feasibility study is being performed. The preliminary results indicate that the therapy is feasible and the conditions for the clinical applications are being set. Another kind of tumor that could be a target for BNCT is osteosarcoma of the limb. The patients that are affected by this tumor have an average age of 19 years, and even if the percentage of cure is about 80%, there are still many patients who have to undergo total limb amputation. Furthermore, due to the infiltrative nature of the tumor, some cells surviving surgery and chemotherapy may give rise to recurrences and pulmonary metastases, which decrease the probability of survival. In this case, being the tumor well localized around the knee, the neutron beam can be relatively small and built at the TRIGA reactor in Pavia.

The BNCT activities at Pavia University are supported by INFN (National Institute for Nuclear Physics), with different experiments for liver (TAOrMINA and ELBA) and lung (WIDEST) and by MIUR (Italian Ministry for University and Research), with 2 PRIN and 2 FIRB projects funded since 2005. Moreover, a research project to test a boron carrier with a Gd group to perform *in vivo* boron quantification by magnetic resonance, is active and it is funded by CARIPLO.

([1]) The research work described in this paper is carried out by a group of Physicists made up of: S. Altieri, F. Ballarini, P. Bruschi, M. A. Gadan, N. Protti, D. Santoro, S. Stella, M. Bonora, F. Borsa, M. Corti. Being a very multi-tasking kind of work, the research is performed together with Biologists: C. Ferrari, L. Cansolino, A. M. Clerici, C. Zonta and Medical Doctors: J. G. Bakeine, A. Zonta.

2. – Boron concentration measurement

The boron concentration measurement is a critical issue in BNCT: the accurate knowledge of boron content in normal and tumor tissue allows to prepare a proper treatment plan, delivering a lethal dose to the tumor and a dose below the tolerance level to the healthy organs. In Pavia, two techniques are currently used: α spectroscopy [9] and neutron autoradiography [10]. The first is a quantitative measurement and exploits the neutron capture reaction occurring in samples of tissues properly prepared and irradiated in the reactor. The frozen samples of organ are cut with a Leica cryostat in 60 μm sections, deposited on mylar disks. The samples are placed inside a rotating device, facing a thin silicon detector. The whole system is under vacuum. The chamber is then irradiated in a position of the thermal column where the thermal neutron flux is about $2 \cdot 10^9\,\mathrm{cm}^{-2}\,\mathrm{s}^{-1}$. A remote control allows to rotate the device, and each sample is irradiated facing the detector tipically for 10 minutes. After this interval of time, the device is rotated and the next sample faces the detector. During the irradiation, the detector collects the charged particles coming from the reactions $^{10}\mathrm{B}(\mathrm{n},\alpha)^7\mathrm{Li}$ and $^{14}\mathrm{N}(\mathrm{n},\mathrm{p})^{14}\mathrm{C}$ and their residual energy spectra are recorded on-line. The obtained spectra have the characteristic shape of absorbed spectra, because the sample thickness is larger than the range of the charged particle in tissue. The boron concentration in the sample irradiated is evaluated analysing the spectrum, in particluar the parts of it where only α-particles contribute, and taking into account the thermal neutron flux, the cross section of the capture, and the efficency of the equipment.

The other technique is a qualitative method to visualize boron bio-distribution in tissue samples. It consists of depositing tissue sections 60 μm thick on CR-39 films which are only sensitive to high LET radiation (SSNTD [11]). The samples on the CR-39 are irradiated in the thermal column of the TRIGA reactor, in the same position as the samples for α spectrometry. The α-particles, the lithium ions and the protons coming from the neutron capture in boron and nitrogen, produce latent tracks in the CR-39. An etching in Na-OH solution makes the tracks visible, creating an image of the boron distribution in the sample: areas of the tissues with higher boron concentration, produce a higher tracks density which results in darker areas in the image. The parameters to be adjusted in order to optimize the images are the neutron fluence, the etching time and temperature, the concentration of the NaOH solution. In order to evaluate the selective uptake in tumor, a neutronigraphic image is compared to a histological preparation of a tissue section cut just after the irradiated one. It is thus possible to correlate the tumor nodules recognizable in the histology to the darker areas present in the CR-39 (fig. 1).

The two described methods are combined in the case of mixed tumor samples, where viable tumor cells, healthy cells and necrosis may be simultaneously present. In this case it would be impossible to separate boron concentration in active tumor cells from boron concentration in normal tissue only by a spectroscopy measurement. Preparing three sections, one for spectrometry, one for auto neutronigraphy and one for histology, it is possible to measure the area occupied by tumor in the sample used for spectroscopy, and to properly weight the contribution of healthy tissue and tumor in the average concentration measured. This is accomplished measuring boron concentration in a healthy, uniform tissue sample and using this value to calculate the boron concentration in tumor.

In the last 2 years, another branch of BNCT research was opened in Pavia. It is in fact well known, that a substantial improvement of BNCT will be reached when boron concentration will be measured *in vivo* by an imaging method such as MRI or PET. To this end, an experiment was started in collaboration with biologists, chemists and

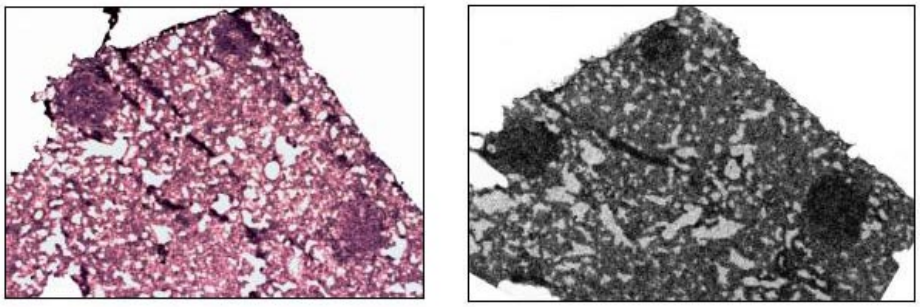

Fig. 1. – Comparison between the histological preparation of a lung section with tumor nodules (right) and the neutronigraphy of a subsequent section (left). The three circular darker structures on the left correspond to the three tumor nodules on the right, demonstrating that tumor cells uptake higher boron concentration with respect to normal tissue.

physicists to create a new BPA molecule bound to a Gd atom. In this way the Gd concentration could be measured by NMR and correlated to boron concentration [12]. The molecule was synthesized and it was proved *in vitro* to be uptaken and to be visible to NMR spectroscopy. The next steps will be a quantitative measurement by NMR on cell cultures and then in rat tissues, using the boron measurement methods already assessed as inter-calibration system.

3. – Liver BNCT

The possibility to uniformly irradiate an organ and selectively hit the tumor cells, represents the only option to cure patients affected by hepatic metastases from colon adenoscarcinoma. In Pavia, an innovative BCNT method was assessed, which foresees the infusion of the patient with a boron carrier (Borophenylalanine, BPA), the explantation of the organ, the irradiation of the liver inside the thermal column of the TRIGA reactor, and the re-implantation of the organ in the patient [13]. A long pre-clinical research was performed in order to assess the boron pharmacokinetics in liver: rats both healthy and bearing tumor were injected with BPA (300 mg/kg) and were sacrificed after different intervals of time. The boron concentration was then measured in samples taken from the rat livers, frozen in liquid nitrogen and cut for the measurement. Using the methods described above, the ratio of boron concentration in tumor and the boron concentration in liver as a function of the time elapsed after BPA infusion was obtained: between 2 and 4 hours, this ratio is maximum, and higher than 4. This is the optimal interval of time to wait after BPA administration, before irradiating the organ. The facility for the organ irradiation was obtained inside the thermal column of the reactor, building a 1 meter long channel with a cross section of $40 \times 20\,\mathrm{cm}^2$ (fig. 2). Two Bismuth screens were placed at the end of the channel to stop the γ radiation coming from the core.

Using this facility, two patients affected by multiple liver metastases were treated in 2001 and 2003 [14, 15]. In both cases the metastases were completely destroyed: a CT scan performed few days after the treatment evidenced that the tumor nodules had been replaced by necrotic tissue. The first patient survived with a good quality of life for 44 months after BNCT, despite the very poor prognosis before BNCT, due to the

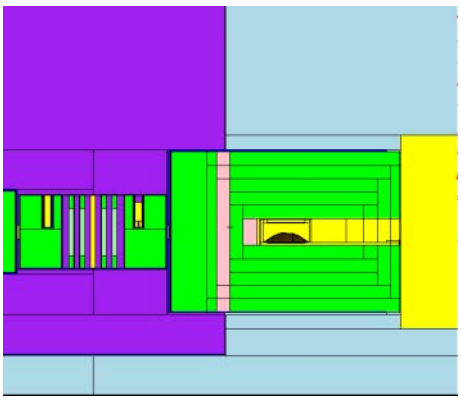

Fig. 2. – (Colour on-line) Section of the irradiation facility obtained in the Thermal Column of the TRIGA reactor, University of Pavia. It is visible the liver placed in the irradiation position inside the channel and the two bismuth screens, protecting it from the γ radiation coming from the core (pink walls between the reactor core and the liver). The reactor was fully simulated by MCNP.

dissemination of the metastases in the whole liver. Then he died for a general recurrency of the tumor in different organs. The second patient, although BNCT succedeed in destroying the metastases, died in 33rd post operative day for a cardiac failure. Obviously two treatments do not allow to draw conclusions as in a complete clinical trial, nevertheless the CT scan of the livers without tumor nodules just after the organ irradiation is a clear demonstration of the BNCT effectiveness in treating tumors that, nowadays, have no other treatment options.

Waiting for other patients to be treated, BNCT research for liver metastases is being carried out measuring boron concentration in human liver samples, in collaboration with the Mainz University, where the liver irradiation is being pursued at their TRIGA reactor [16]. A protocol is active at the University Hospital of Mainz, which allows to administrate BPA to patients who have to undergo partial resection of the liver. After surgery, the portion of the liver which is removed is analyzed by neutron autoradiography, ICP-OES [17], PGNAA [18], and α spectrometry to measure boron uptake in human tumor and liver. This activity represents an important step in the field of BNCT because for the first time, an intercomparison of boron measurement methods is being performed on human tissues. The preliminary results show a substantial agreement between the different methods used.

4. – Lung BNCT

The other branch of the BNCT research in Pavia is dedicated to lung tumors, which are one of the major causes of death for cancer in the Western Countries [19]. In many cases, the lung is invaded by metastases or disseminated tumors which cannot be removed, and also chemotherapy is often not effective. The lung tissue has a density about three times lower than other soft tissues, as liver, and this makes it possible to conceive a BNCT treatment without explantation. Irradiating with epithermal external neutron beams it is possible to obtain a uniform thermal neutron field inside the organ. To demonstrate this, a set of Monte Carlo simulations was performed, using an antropomorphic model named ADAM, kindly provided by ENEA, Bologna [20], and the neutron transport code

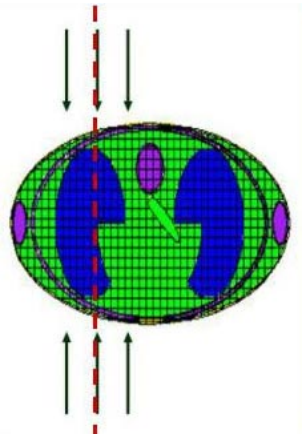

Fig. 3. – MCNP transversal view of ADAM's thorax, with the two ideal collimated epithermal beams for lung BNCT irradiation. The dashed line indicates the longitudinal axis of the lung, along which the uniformity of the thermal neutron field was studied.

MCNP.4c2 [21]. ADAM's thorax was divided into 1 cm^3 voxels and the simulated source consisted of 2 collimated, epithermal (1 keV) opposite neutron beams (antero-posterior and postero-anterior) as shown in fig. 3. This ideal set-up was used to demonstrate the possibility to irradiate uniformly the whole lung, allowing to fully exploit the potentiality of BNCT: the tumor receives a lethal dose wherever it may be, while the normal tissue is not seriously damaged. Assuming a boron concentration ratio between tumor and healthy tissue of 3 [22], the ratio of the biological weighted dose absorbed by tumor and the one absorbed by lung is still about 3. This means that, in principle, it is possible to perform BNCT using external beams to treat tumors disseminated in the lung.

The feasibility of BNCT depends obiously on the boron concentration ratio obtained between tumor and lung. To study this issue, a number of rats, both healthy and bearing lung metastases, were injected with BPA and sacrificed after different interval of times. Their lungs were taken, frozen in liquid nitrogen, and prepared for boron measurements by α spectroscopy and neutron autoradiography. As anticipated before, from 4 hours after BPA administration, the boron concentration ratio remains higher than 3 up to 8 hours. This result, together with the possibility to obtain a uniform thermal neutron field inside the lung, encourages to go on with the research.

The next step is the *in vivo* effectiveness study, to test the capacity of BNCT to destroy the metastases and to evaluate its effects both on tumor and on normal tissue. Some rats will be irradiated in the thermal column of the TRIGA reactor, in a proper shielding with a window for lung irradiation. The rats will be divided into 3 groups: a control group with lung metastases which will not receive any treatment, a group with metastases which will be irradiated without boron and a third group which will receive the entire BNCT treatment. The shielding was studied by means of Monte Carlo calculations [23]. Presently, the shielding box is available and all the permissions to irradiate the animals at the reactor have been obtained.

5. – Osteosarcoma BNCT

Osteosarcoma is a highly malignant tumor constituted by malignant mesenchymal cells which produce osteoid and bone tissue. It is the most common primary malignant

tumor of the skeleton with an average age of the patients of about 19 years. The global mean survival at 5 years is 55–70%. However, when pulmonary metastases occur, the survival is reduced to less than 30% in 80% of the cases. Usually, osteosarcoma is treated by surgery combined to chemotherapy. In 80% of cases the operation is limb salvage (without amputation), but when the tumor affects also vessels and nerves, the limb amputation is still necessary. BNCT could be a valid option in this field, because, after finding a good carrier of boron able to enrich osteosarcoma cells, its effectiveness lies on high-LET radiation. Moreover, it would be possible to irradiate with a proper beam a larger part of the limb, to decrease the probability that some cells could survive to the surgery. A project for BNCT of osteosarcoma was recently funded by the Italian Ministry of University and Research in the program *FIRB-Futuro in Ricerca*. It is aimed to: verify the osteosarcoma BNCT feasibility, establish the conditions for the clinical applications of BNCT for the limb osteosarcoma, and complete the pre-clinical research and the design of the patient irradiation facility at the TRIGA reactor in Pavia. Not only this research is ment to contribute to the cure of limb osteosarcoma, but also to increase the effectiveness of BNCT developing new boron carriers. In fact, the project was conceived in collaboration between Pavia and Florence University, where a group of chemists will formulate and characterize new boronated compounds that will be tested both *in vitro* and *in vivo* in Pavia. The first measurements of boron concentration in cells cultured in presence of BPA have already been performed, proving that also osteosarcoma cells uptake boron if vehiculated by BPA [24].

6. – Conclusions

Boron Neutron Capture Therapy is a highly multi-tasking research involving physicists, biologists, chemists and medical doctors. The state of the art is that BNCT is still an experimental radiotherapic technique, and some work has still to be done before it can be considered a conventional therapy. Considerable improvements in this direction will be reached when carriers able to concentrate boron in the tumor much more than BPA will be formulated. Moreover, it will be necessary to measure boron concentration *in-vivo* by an imaging method just before the neutron irradiation. These issues are being addressed in Pavia, in the framework of BNCT research for liver and lung metastases and for limb osteosarcoma.

REFERENCES

[1] Barth R., Coderre J., Vicente M. G. and Blue T. E., *Clin. Cancer Res.*, **11** (2005) 3987.

[2] Kankaanranta L., Seppl T., Koivunoro H., Saarilahti K., Atula T., Collan J., Salli E., Kortesniemi M., Uusi-Simola J., Mkitie A., Seppnen M., Minn H., Kotiluoto P., Auterinen I., Savolainen S., Kouri M. and Joensuu H., *Int. J. Radiat. Oncol. Biol. Phys.*, **69** (2007) 475.

[3] Miyatake S., Kawabata S., Yokoyama K., Kuroiwa T., Michiue H., Sakurai Y., Kumada H., Suzuki M., Maruhashi A., Kirihata M. and Ono K., *Appl. Radiat. Isot.*, **67** (2009) S15.

[4] Menndez P. R., Roth B. M. C., Pereira M. D., Casal M. R., Gonzlez S. J., Feld D. B., Santa Cruz G. A., Kessler J., Longhino J., Blaumann H., Jimnez Rebagliati R., Calzetta Larrieu O. A., Fernndez C., Nievas S. I. and Liberman S. J., *Appl. Radiat. Isot.*, **67** (2009) S50.

[5] Suzuki M., Sakuray Y., Masunaga S., Kinashi Y., Nagata K., Maruhashi M. and Ono K., *Int. J. Radiat. Oncol. Biol. Phys.*, **66** (2006) 1523.
[6] Trivillin V. A., Heber E. M., Nigg D. W., Itoiz M. E., Calzetta O., Blaumann H., Longhino J. and Schwint A. E., *Rad. Res.*, **166** (2006) 387.
[7] Suzuki M., Sakurai M., Hargiwara S., Masunaga S., Kinashi Y., Nagata K., Murahashi A., Kudo M. and Ono K., *Jpn. J. Clin. Oncol.*, **37** (2007) 376.
[8] Mitin V. N., Kulakov V. N., Khokhlov V. F., Sheino I. N., Arnopolskaya A. M., Kozlovskaya N. G., Zaitsev K. N. and Portnov A. A., *Appl. Radiat. Isot.*, **67** (2009) S299.
[9] Wittig A., Michel J., Moss R. L., Stecher-Rasmussen F., Arlinghaus H. F., Bendel P., Mauri P. L., Altieri S., Hilger R., Salvadori P. A., Menichetti L., Zamenhof R. and Sauerwein W. A. G., *Crit. Rev. Oncol. Hematol.*, **68** (2008) 66.
[10] Altieri S., Bortolussi S., Bruschi P., Chiari P., Fossati F., Stella S., Prati U., Roveda L., Zonta A., Zonta C., Ferrari C., Clerici A., Nano R. and Pinelli T., *Appl. Radiat. Isot.*, **66** (2008) 1850.
[11] Griffith R. V. and Tommasino L., in *The Dosimetry of Ionising Radiations*, Vol. **III**, edited by Kase K., Bjargard B. B. and Attix F. H. (Academic Press, New York) 1991, pp. 323-426.
[12] Takahashi K., Nakamura H., Furumoto S., Yamamoto K., Fukuda H., Matsumura A. and Yamamoto Y., *Bioorganic Med. Chem.*, **13** (2005) 735.
[13] Pinelli T., Zonta A., Altieri S., Barni S., Braghieri A., Pedroni P., Bruschi P., Chiari P., Ferrari C. and Zonta C., *TAOrMINA: from the first idea to the application to the human liver* in *Research and Development in Neutron Capture Therapy*, edited by Sauerwein W., Moss R. and Wittig A. (Monduzzi Editore, Bologna, Italy) 2002, pp. 1065-1072.
[14] Zonta A., Prati U., Roveda L., Ferrari C., Zonta S., Clerici A. M., Zonta C., Bruschi P., Nano R., Barni S., Pinelli T., Fossati F., Altieri S., Bortolussi S., Chiari P. and Mazzini G., *J. Phys. Conf. Ser.*, **41** (2006) 484.
[15] Zonta A., Pinelli T., Prati U., Roveda L., Ferrari C., Clerici A. M., Zonta C., Mazzini G., Dionigi P., Altieri S., Bortolussi S., Bruschi P. and Fossati F., *Appl. Radiat. Isot.*, **67** (2009) S67.
[16] Hampel G., Wortmann B., Blaickner M., Knorr J., Kratz J. V., Lizn Aguilar A., Minouchehr S., Nagels S., Otto G., Schmidberger H., Schtz C. and Vogtlnder L., *Appl. Radiat. Isot.*, **67** (2009) S238.
[17] Probst T. U., *Fresenius J. Anal. Chem.*, **364** (1999) 391.
[18] Paul R. L. and Lindstrom R. M., *J. Radioanal. Nucl. Chem.*, **243** (2000) 181.
[19] Jemal A., Murray T., Ward E., Samuels A., Tiwari R. C., Ghafoor A., Feuer E. J. and Thun M. J., *Cancer J. Clin.*, **55** (2005) 10.
[20] Altieri S., Bortolussi S., Bruschi P., Chiari P., Fossati F., Facoetti A., Nano R., Clerici A. M., Ferrari C., Zonta A., Zonta C., Marchetti A., Solcia E., Bakeine J. G. and Salvucci O., in *Advances in Neutron Capture Therapy*, edited by Nakagawa Y., Kobayashi T. and Fukuda H. (Takamatsu, Japan) 2006, pp. 500-503.
[21] Briesmeister J. F., *A general Monte Carlo N-particle transport code, version 4C, LA-13709-M* (2000).
[22] Bortolussi S., Bakeine J. G., Ballarini F., Bruschi P., Gadan M. A., Protti N., Stella S., Clerici A. M., Ferrari C., Cansolino L., Zonta C., Zonta A., Nano R. and Altieri S., *Appl. Radiat. Isot.*, **69** (2011) 394.
[23] Protti N., Bortolussi S., Stella S., Gadan M. A., De Bari A., Ballarini F., Bruschi P., Ferrari C., Clerici A. M., Zonta C., Bakeine J. G., Dionigi P., Zonta A. and Altieri S., *Appl. Radiat. Isot.*, **67** (2009) S210.
[24] Ferrari C., Zonta C., Cansolino L., Clerici A. M., Gaspari A., Altieri S., Bortolussi S., Stella S., Bruschi P., Dionigi P. and Zonta A., *Appl. Radiat. Isot.*, **67** (2009) S341.