Applied Radiation and Isotopes 104 (2015) 155–159

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

Application of BNCT to the treatment of HER2+ breast cancer recurrences: Research and developments in Argentina

M.A. Gadan [a,*], S.J. González [a,b], M. Batalla [a], M.S. Olivera [a], L. Policastro [a,b], M.L. Sztejnberg [a]

[a] National Atomic Energy Commission (CNEA), Avenida Del Libertador, 8250 Ciudad de Buenos Aires, Argentina
[b] National Council of Scientific and Technological Research (CONICET), Avenida Rivadavia, 1970 Ciudad de Buenos Aires, Argentina

HIGHLIGHTS

- A new proposal of BNCT for HER2+ breast cancer treatment is introduced.
- The proposal considers development of immunoliposomes as boron carrier nanovehicles.
- Locoregional recurrences after treatment were identified as candidates for initial BNCT studies.
- First analysis show acceptable neutron flux distributions provided by RA-6 BNCT facility.

ARTICLE INFO

Article history:
Received 7 February 2015
Received in revised form
13 May 2015
Accepted 2 July 2015
Available online 3 July 2015

Keywords:
HER2-enriched breast cancer
Post treatment recurrences
Immunoliposomes

ABSTRACT

In the frame of the Argentine BNCT Project a new research line has been started to study the application of BNCT to the treatment of locoregional recurrences of HER2+ breast cancer subtype. Based on former studies, the strategy considers the use of immunoliposomes as boron carriers nanovehicles to target HER2 overexpressing cells. The essential concerns of the current stage of this proposal are the development of carriers that can improve the efficiency of delivery of boron compounds and the dosimetric assessment of treatment feasibility. For this purpose, an specific pool of clinical cases that can benefit from this application was determined. In this work, we present the proposal and the advances related to the different stages of current research.

1. Introduction

HER2-enriched breast cancer subtype (HER2+) is characterized by overexpression of HER2 transmembrane protein. This macromolecule is normally expressed in healthy cells and is related to regulation of cell growth and differentiation. As it has been determined from clinical studies this breast cancer subtype is associated to reduced overall survivals and short times to relapse, thus associated to a poor prognosis to patients (Slamon et al., 1989; Yarden and Sliwkowski, 2001).

Protein overexpression allows treatment with targeted therapy by administration of anti-HER2 monoclonal antibodies. Trastuzumab, a humanized monoclonal antibody, is currently the most commonly used for this purpose. However, a group of patients do not show a satisfactory response to this treatment and, consequently, new research focuses in different strategies to overcome this situation, including the development of new monoclonal antibodies (Valabrega et al., 2007; Pohlmann et al., 2009). In Argentina, accordinsg to statistical data derived from the 'National HER2 test Program', the prevalence of HER2+ breast cancer is 13.2% from a population of 34,640 cases analyzed between 2005 and 2010 (Cáceres et al., 2012).

A new research line stimulated by previous studies (Bohl Kullberg et al., 2005; Mundy et al., 2006; Sztejnberg and Jevremovic, 2009) has been started in the National Atomic Energy Commission as part of the Argentine BNCT Project. This research line aims at addressing certain selected cases of those resilient HER2+ breast cancers that oncologists would consider an appropriate match for BNCT.

Our proposal consists of the application of BNCT using immunoliposomes as boron carrier nanovehicles to target HER2 overexpressing cells. Immunoliposomes labeled with the monoclonal antibody trastuzumab results in specific binding to HER2 overexpressing breast cancer cells and would allow to enhance both selectivity and concentration levels of boron compounds in tumor microenvironment.

* Correspondence to: Presbítero Luis González y Aragón 15, zip code B1802AYA, Ezeiza, Buenos Aires, Argentina.
E-mail address: mgadan@cae.cnea.gov.ar (M.A. Gadan).

http://dx.doi.org/10.1016/j.apradiso.2015.07.006
0969-8043/

The essential concerns of this stage of the proposal are: (a) identification of specific pool of clinical cases that could benefit from a potential application of BNCT; (b) development of boron nanocarriers that could improve the selectivity of boron compounds; and (c) feasibility assessment from dosimetric point of view aimed at evaluating biodistribution requirements and irradiation conditions taking into account the identified clinical cases.

This work introduces CNEA's new research line together with the determination of clinical cases currently being considered for the feasibility study for the application of BNCT, advances in the development of liposomes and some initial considerations regarding the dosimetric analysis.

2. Clinical cases

The study of application of BNCT to the treatment of HER2+ breast cancer includes among its objectives to establish the conditions for which this therapy can be offered. Special focus is paid to cases that do not satisfactorily respond to current therapeutics or whether BNCT would mean a lower morbidity. Considering this scenario and based on their practical experience, local medical doctors have identified as general selection criteria those locoregional recurrences that may arise after breast cancer treatment as candidate cases (Gadan, 2014). From surgical treatment standpoint, locoregional recurrences can be classified as arising after breast conserving therapy (BCT) or after mastectomy. Local presentation of recurrences after BCT includes the ipsilateral breast (which stands for the treated breast) and the surgery scar. Local presentation after mastectomy concerns mastectomy bed and surgery scar too. Regional recurrence presentation concerns axillary and supraclavicular lymph nodes independently from the surgery treatment applied (Moran et al., 2014).

Regarding clinical presentation, local relapses after BCT can affect the glandular parenchyma but can also involve the skin. This later condition is associated with an increased risk of progression to metastatic disease. The relapse rate in patients treated on local medical institutions varies between 9% and 10.2% (Núñez de Pierro et al., 2004; Ghiraldo et al., 2012). The most common site for post-mastectomy local relapse is the mastectomy bed of the ipsilateral chest wall, either skin or sub-dermal deposits, and may occur not only as a single phenomenon but also may indicate the development of distant metastases (Buchanan et al., 2006). The relapse rate on local medical institutions can vary between 3 and 5%. Regional recurrences presentation is rare compared to local relapse. Patients with this type of recurrence have a poor prognosis because they are linked to a higher chance of developing distant metastases. In Argentina, some retrospective studies report a rate of regional relapse of 0.7% with an expectation range between 0.3% and 2% (Núñez de Pierro et al., 2004).

Prognosis for patients with local recurrence after BCT is better than that of patients with chest wall recurrence after mastectomy. Radical mastectomy is the treatment of choice for this relapses. In the same way, the primary local treatment for post-mastectomy chest wall recurrence is the surgery. Alternatively, systemic therapy is administered only for control of a potential spread of the disease. Concerning regional recurrences, since they are not a frequent event, the possibility of establishing an optimal management of the disease is difficult and varies according to medical institution criteria (Pedersen et al., 2011). In general, attention should be paid to patients that have already received radiotherapy for the treatment of primary tumor since the first radiotherapy treatment limits future irradiations. A new application of radiotherapy could compromise the already irradiated healthy tissues, presenting higher morbidity. Reducing the amount of fields and controlling the total dose to avoid toxicity effects do not allow adequate treatment of the disease.

Considering these aspects, cases of patients who relapse after primary breast cancer treatment represent a group with a real need for treatment for which the application of BNCT could mean a potential benefit. These cases should be addressed and be included in the feasibility analysis of this proposal treatment.

3. Liposomes

Current work also involves the evaluation of boron–phenylalanine–fructose (BPA–Fru) encapsulation in liposome testing different lipids formulations. Neutral charge liposomes were obtained by the combination of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), cholesterol and phosphoethanolamine-[methoxy(polyethyl-eneglycol)-2000 (DSPE-PEG) at 61.8:37:1.2 M ratio respectively, whereas cationic liposomes were obtained adding 3.7% of dimethyldioctadecylammonium bromide (DDAB) to the mentioned formulation (60.5:36.3:1.25:1.95). Liposomes were performed using the lipid film hydration technique. Briefly, lipids were previously dissolved in chloroform (Mallinckrodt Baker, Inc., Paris, KY) and dried under a steady stream of nitrogen for 2 h. Films were hydrated with a solution of 8 mg/ml BPA–Fru in phosphate buffered saline (PBS). Multilamellar liposomes were subsequently extruded through 800 nm, 400 nm, 200 nm and 100 nm polycarbonate membranes using an extruder (Avanti Polar Lipids, USA). Subsequently, unilamellar liposomes were purified by size exclusion chromatography column (Sepharose CL-4B). Aliquots that contain liposomes were identified by absorbance measurements at two wavelengths (265 nm and 280 nm) using a UV–vis spectrophotometer (Nanodrop 2000 UV, Thermo Scientific) and were concentrated using 10,000 MWC concentrators (VivaSpin6, GE Healthcare). Mean size distribution and polydispersity index (PI) of liposomes were determined by dynamic light scattering (Zetasizer Nano; Malvern, UK).

Encapsulation efficiency of different lipids formulations was evaluated by determination of the boron to phosphorus ratio (B/P). In order to obtain boron concentration, samples were digested and appropriately diluted for subsequent measurement of boron by Optical Inductive Couple Plasma (ICP-OES, Jobin Yvon). To determine phosphorus concentration, phospholipids were previously extracted by Bligh and Dyer protocol and phosphorus was quantified by Bartlett-modified method (Bartlett, 1959).

Neutral charge liposomes size distribution has a mean value of 127 ± 25 nm with a polidispersity index (PI) value 0.187 while cationic liposomes size distribution has a mean value of 120 ± 20 nm with PI value 0.177. The B/P ratio of neutral charge liposomes was 0.2, while an increment of 65% of B/P ratio was achieved in cationic with respect to neutral liposomes formulation.

4. Neutron flux study and dosimetry considerations

The dosimetric feasibility assessment considers as a first option the use of existing irradiation facilities in our country, such as the new RA-6 BNCT facility. This facility has been designed for the treatment of cutaneous melanoma (Longhino and Blaumann, 2010). The new beam, named B2, has a mixed thermal and epithermal composition, which allows a deeper penetration in tissue compared to a pure thermal beam, and a low fast neutron component.

As a starting point in this part of the research, numerical characterizations of the beam and neutron flux distribution were performed using anthropomorphic computational models, taking into account the selected clinical cases and an a neutron beam

impinging "en face" (as recommended in Horiguchi et al. (2011), for mastectomy cases). Since selected clinical cases were based on general criteria selection and not in individualized patients, at this starting stage, it was established to work using computational models allowing to study different organs volumes without real patient's CT scans. Future work will involve dosimetric study based on CT scans from real patients.

Breast volume has direct implications on neutron flux distribution; thus for post BCT local relapse cases, breast volumes of 300 ml (*BCT300*) and 600 ml (*BCT600*) were considered according to the breast average volume reported in different publications (Kovacs et al., 2007; Kayar et al., 2011). Post mastectomy local relapse considers the case of a left side mastectomy and a right breast volume of 300 ml (*MAST300*) was proposed. The latter case is virtually a relapse in skin similar to cutaneous melanoma in terms of anatomical depth presentation condition. For all the models the recurrence was anatomically located at different depths on the left side which is a more unfavorable condition considering heart irradiation and exposure to healthy organs at risk. Computer simulations were performed using particle transport code MCNP5 (X-5 Monte Carlo Team, 2003) and the track-by-track computational source (rssa) of RA-6 reactor irradiation facility (Longhino and Blaumann, 2010). The anthropomorphic models were built utilizing the XCAT phantom generator code (Segars et al., 2010). Normalized neutron flux distribution for both thermal ($E < 0.5$ eV) and fast ($E > 10$ keV) components are shown for *MAST300* (Fig. 1). Normalization is referred to the maximum value of thermal component. The fast neutron contribution is globally small and less than 2% in all of the studied cases. This is a desirable condition since tissue KERMA coefficients for fast neutrons

can be larger than those for thermal neutrons, even for boron in typical concentrations (Sztejnberg and Jevremovic, 2009). Thus they can significantly contribute to a non-therapeutic dose and, consequently, must be minimized.

A simple estimate of the impact of the flux distribution to dosimetry can be made linking the boron dose in tumor to boron dose in different organs at risk and neglecting—only for the moment—the rest of the dose components, according to the following boron dose ratio expression (1):

$$R_B = \frac{D^B_{min,\ tumor}}{D^B_{MAX,\ normal}} \sim \frac{\varphi_{th,min,tumor}}{\varphi_{th,MAX,normal}} \cdot \frac{B^{10}_{tumor}}{B^{10}_{normal}} \quad (1)$$

where $\varphi_{\mathrm{nth\ min\ tumor}}$ and $\varphi_{\mathrm{nth\ max\ normal}}$ are the minimum and

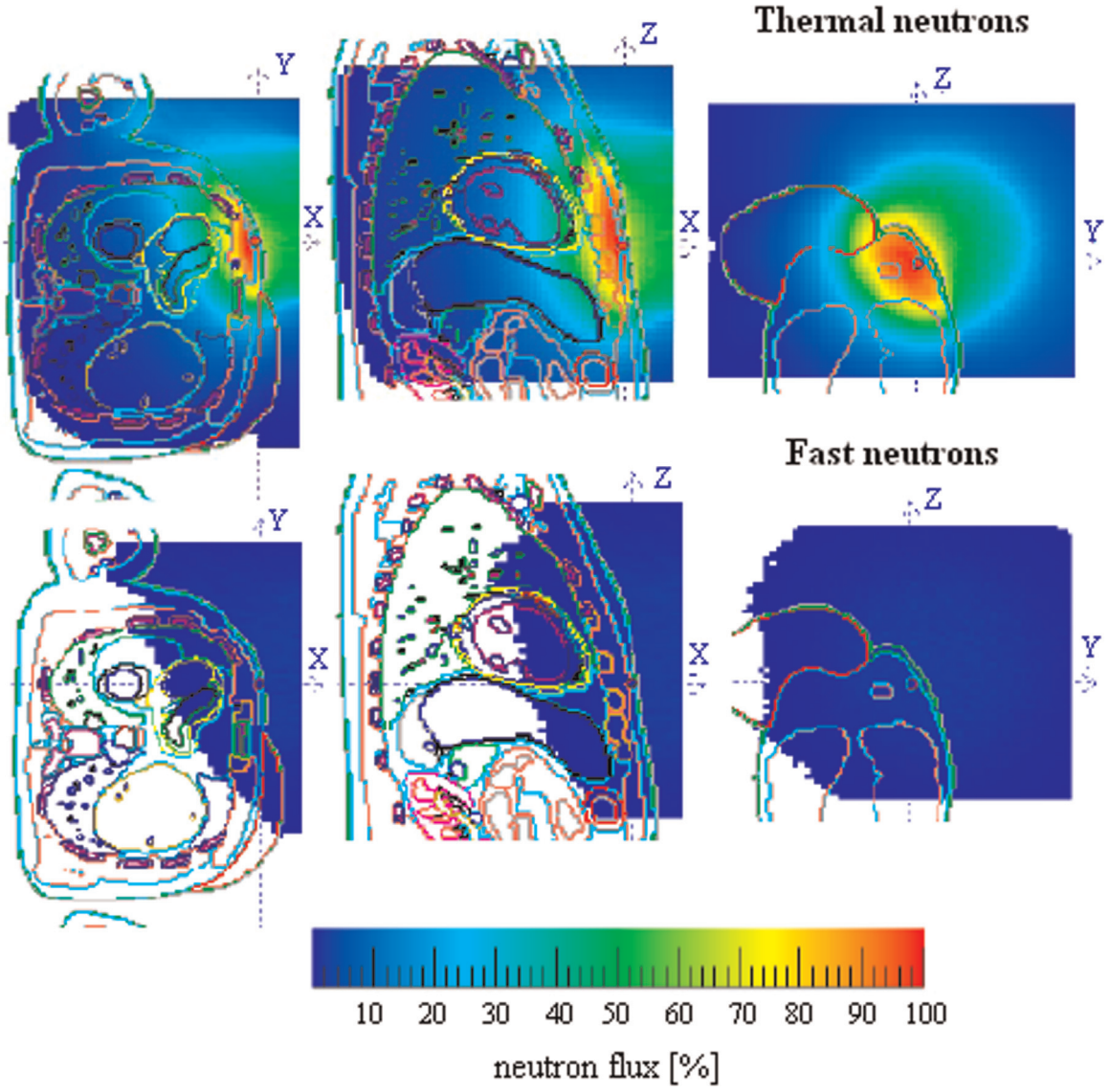

Fig. 1. Relative neutron flux distribution for both thermal and fast components for the analyzed post mastectomy recurrence clinical case (Visualization Moritz ver. 1.25).

Table 1
Boron dose ratios obtained according to (1) for normal tissues in different proposed models.

Normal tissue/case study	BCT600	BCT300	MAST300
Skin	1.4	1.7	1.7
Heart	7.5	5.1	4.2
Lungs	5.6	4.3	3.9

maximum value of thermal neutron flux in tumor and normal tissue, respectively; and $B^{10}{}_{tumor}$ and $B^{10}{}_{normal}$ are their corresponding boron concentrations. Table 1 lists the obtained boron dose ratios (R_B) considering boron concentration ratios, $B^{10}{}_{tumor}$: $B^{10}{}_{normal}$, of 3:1 for heart and lung and 3:1.8 for skin (Fukuda et al., 1999).

Fig. 1 and the values in Table 1 show that thermal neutron flux in tumor is very close to or lower than the same neutron component reached in skin; and this means that the therapeutic selectivity would be given by the boron compound. Calculated ratios show that these organs would receive boron-induced doses smaller than the tumor. At this point, dose ratios seem to be large enough to deliver large enough doses to tumor while sparing healthy tissue from complications.

The reported maximum-to-minimum values for MAST300 coincide with the maximum-to-minimum dose ratio calculated from the work of Horiguchi et al. (2011), where effectiveness factors are included. The largest difference is for heart where tumor-to-heart CBE ratio plays a great role. Since (boron) dose delivered to skin is the closest to tumor (boron) dose, skin might be the organ at risk limiting the neutron irradiation. Given the favorable ratios, significantly larger than one-even in BCT600 case, neutron flux seems to be appropriately tailored and moderated.

Dose prescriptions to tumor in conventional radiotherapy of the breast are typically ~30 Gy (Cutuli et al., 2005; Horiguchi et al., 2011). The doses to healthy tissue should be below 24 Gy for skin (secondary ulceration), 11 Gy for 2/3 of the lung (radiation pneumonitis)[1], and 12 Gy for whole heart[2] (Schultz-Hector and Trott, 2007; ICRP, 2012).[3] The therapeutic ratios should be larger than 1.3 for skin, 2.7 for lungs, and 2.5 for heart, what is accomplished by values in Table 1, with the utilized boron concentration ratios. Still, more stringent conditions might be useful for greatly improving outcomes obtained with already established modalities.

This is a first approach that shows the bottom-line conditions in terms of the required boron concentration and available irradiation facilities. It is still necessary to conduct more detailed analysis including other dose contributions, effectiveness factors, tolerance limits, and dose-volume studies. This assessment is strongly linked to the performance required from the immunoliposomes proposed as vehicles of boron. This delivery system is being investigated in order to achieve the conditions that optimize the efficiency and selectivity of boron delivery and to achieve better boron concentrations and concentration ratios than those taken as reference in these calculations.

[1] A dose of 6.5 Gy is considered for whole lung, which is scaled up for 2/3 of the volume (according to Fig. 1) from relations found in Emami et al. (1991).

[2] Heart tolerance dose might be a troublesome value since it is some of the less defined values. It used to be considered one of the most radioresistant organs but there are observations that might mean something very different. Symptomatology is many times not directly correlated to irradiation, involving a variety of late effects, and dose-effect paradigm is confounding. Nevertheless, radiation damage appearance probability seems to be proportional to delivered dose and not directly correlates to a threshold. The mentioned value is typically accepted for treatments and reflects appearances of cardiopathies after treatments such as for breast cancer and Hodgkin's lymphoma.

[3] These are dose values for single fraction/acute irradiations. Equivalence from multi-fraction treatments was calculated, where necessary, though linear-quadratic model with standard parameters.

5. Conclusions

Identified clinical cases includes locoregional recurrences after breast cancer treatment that can arise as local in ipsilateral breast or mastectomy bed, and regional as axillary or supraclavicular presentation. The current definition does not exclude future inclusion of other clinical recurrences that currently have no effective treatment and can benefit from the application of BNCT.

Boron containing liposomes were prepared achieving higher encapsulation efficiency with cationic ones. In order to enhance boron concentration in liposomes current work directions include development of cationic liposomes encapsulating boron compounds other than BPA-Fru.

A first approach to dosimetry was performed that shows that the thermal neutron flux distributions achieve acceptable conformation for different proposed cases, the skin being the principal limiting organ at risk. The next stage of work will involve a more detailed analysis considering specific macrodosimetric dose parameters and different boron distribution scenarios.

Acknowledgements

Special thanks to Dr. Allemand and Dr. Nuñez de Pierro for all fruitful discussions pointed to the identification of clinical cases and for the support to the development of this proposal. The authors thanks to Dr. Paul Segars for kindly providing XCAT software.

This work was partially financed by Proyecto de Investigación Científica y Tecnológica PICT-2012-1590 conferred by Agencia Nacional de Promoción Científica y Tecnológica.

References

Bartlett, G.R., 1959. Phosphorus assay in column chromatography. J. Biol. Chem. 234, 466–468.

Bohl Kullberg, E., Wei, Q., Capala, J., Giusti, V., Malmstrom, P., Gedda, L., 2005. EGF-receptor targeted liposomes with boronated acridine: growth inhibition of cultured glioma cells after neutron irradiation. Int. J. Radiat. Biol. 81 (8), 621–629.

Buchanan, C.L., Dorn, P.G., Fey, J., Giron, G., Naik, A., Mendez, J., Murphy, C., Sclafani, L.M., 2006. Locoregional recurrence after mastectomy: incidence and outcomes. J. Am. Coll. Surg 203 (4), 469–474.

Cáceres, V., Acosta, G., Sarancone, S., Arra, A., Paradelo, M., López Presas, A., Bravo, I., Martins, D., Marraco, G., Lago, N., Calafat, P., Mosto, J., Zoppi, J., Glatstein, T., Urbano Martínez, M., Palazzi, J., Columbo, S., Frahm, I., 2012. Programa nacional de diagnóstico de la sobreexpresión de HER2 en pacientes con cáncer de mama. Rev. Arg. Mastol. 31 (110), 8–22.

Cutuli, B., de Lafontan, B., Quentin, P., Mery, E., 2005. Breast-conserving surgery and radiotherapy: a possible treatment for lobular carcinoma in situ? Eur. J. Cancer 41 (3), 380–385.

Emami, B., Lyman, J., Brown, A., Coia, L., Goitein, M., Munzenrider, J.E., Shank, B., Solin, L.J., Wesson, M., 1991. Tolerance of normal tissue to therapeutic irradiation. Int. J. Oncol. Biol. Phys. 21, 109–122.

Fukuda, H., Honda, C., Wadabayashi, N., Kobayashi, T., Yoshino, K., Hiratsuka, J., Takahashi, J., Akaizawa, T., Abe, Y., Ichihashi, M., Mishima, Y., 1999. Pharmacokinetics of 10B-p-boronophenylalanine in tumor, skin and blood of melanoma patients: a study of boron neutron capture therapy for malignant melanoma. Melanoma Res. 9 (1), 75–83.

Gadan, M.A., 2014. Determinación de casos clínicos para el estudio de aplicación de BNCT para el tratamiento de cáncer de mama HER2 CNEA. Internal Report IN-06Y-135.

Ghiraldo, L., Elizalde, R.J., Newman, M., Schiavi, C., Recaman, N., Alexenicer, C., Solucci, C., Jaroslavsky, M.J., 2012. Factores asociados a recidiva en tratamiento conservador del cáncer de mama invasivo. Rev. Arg. Mastol. 31 (111), 147–158.

Horiguchi, H., Nakamura, T., Kumada, H., Yanagie, H., Suzuki, M., Sagawa, H., 2011. Investigation of irradiation conditions for recurrent breast cancer in JRR-4. Appl. Radiat. Isot. 69 (12), 1882–1884.

ICRP, 2012. ICRP statement on tissue reactions/early and late effects on radiation in normal tissue and organs – threshold doses for tissue reactions in a radiation protection context. ICRP Publication 118 Ann. ICRP 41 (1/2).

Kayar, R., Civelek, S., Cobanoglu, M., Gungor, O., Catal, H., Emiroglu, M., 2011. Five methods of breast volume measurement: a comparative study of measurements of specimen volume in 30 mastectomy cases. Breast Cancer: Basic Clin. Res. 5, 43–52.

Kovacs, L., Eder, M., Hollweck, R., Zimmermann, A., Settles, M., Schneider, A., Endlich, M., Mueller, A., Schwenzer-Zimmerer, K., Papadopulos, N., Biemer, E., 2007. Comparison between breast volume measurement using 3D surface imaging and classical techniques. The Breast 16, 137–145.

Longhino, J.M., Blaumann, H.R., 2010. Current status of the RA-6 BNCT facility. In: Liberman, S., Kreiner, A., (Eds.), New Challenges in Neutron Capture Therapy. Proceedings of 14th International Congress on Neutron Capture Therapy. Buenos Aires, Argentina, pp. 391–394.

Moran, M.S., Chagpar, A.B., Mayer, E.L., 2014. Diseases of the Breast, fifth ed. Wolters Kluwer Health, Philadelphia, pp. 891–904.

Mundy, D., Harb, W., Jevremovic, T., 2006. Radiation binary targeted therapy for HER-2 positive breast cancers: assumptions, theoretical assessment and future directions. Phys. Med. Biol. 51, 1377–1391.

Núñez de Pierro, A., Allemand, D., Agejas, G., 2004. Tratamiento conservador del cáncer de mama. Evaluación de los resultados en 1306 pacientes. Experiencia del grupo de trabajo del Hospital Juan A. Fernández de Buenos Aires. Rev. Arg. Mastol. 23 (78), 69–93.

Pedersen, A.N., Møller, S., Steffensen, K., Haar, V., Jensen, M., Kempel, M.M., Jepsen, S.L., Madsen, E.L., Roslind, A., Sandberg, E., Schöllkopf, C., Sørensen, P., Windfeldt, K.M., Andersson, M., 2011. Supraclavicular recurrence after early breast carcinoma: a curable condition? Breast Cancer Res. Treat. 125, 815–822.

Pohlmann, P.R., Mayer, I.A., Mernaugh, R., 2009. Resistance to trastuzumab in breast cancer. Clin. Cancer Res. 15, 7479–7491.

Segars, P., Sturgeon, G., Mendonca, S., Grimes, J., Tsui, B.M., 2010. 4D XCAT phantom for multimodality imaging research. Med. Phys. 37 (9), 4902–4915.

Schultz-Hector, S., Trott, K.R., 2007. Radiation-induced cardiovascular diseases: is the epidemiologic evidence compatible with the radiobiologic data? Int. J. Radiat. Oncol. Biol. Phys. 67 (1), 10–18.

Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A., Press, M.F., 1989. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Sci. New Ser. 244 (4905), 707–712.

Sztejnberg, M.L., Jevremovic, T., July 2009. Advanced applications of BNCT in advanced cancers. In: Proceedings of 17th ASME International Conference on Nuclear Engineering, vol. 2, Brussels, pp. 905–914.

Valabrega, G., Montemurro, F., Aglietta, M., 2007. Trastuzumab: mechanism of action, resistance and future perspectives in HER2-overexpressing breast cancer. Ann. Oncol. 18 (6), 977–984.

X-5 Monte Carlo Team, 2003. MCNP—A General Monte Carlo N-Particle Transport Code Version 5 (Revised 2008). Los Alamos National Laboratory.

Yarden, Y., Sliwkowski, M.X., 2001. Untangling the ErbB signalling network. Nat. Rev. Mol. Cell Biol. 2, 127–137.