Tumour bed irradiation of human tumour xenografts in a nude rat model using a common X-ray tube

S V Tokalov*, W Enghardt and N Abolmaali

OncoRay – Centre for Radiation Research in Oncology, Medical Faculty Carl Gustav Carus, TU Dresden, Fetscherstr. 74, 01307 Dresden, Germany

**Corresponding author (Fax, +49 351 458 7311; Email, Sergey.Tokalov@oncoray.de)*

Studies that investigate the radiation of human tumour xenografts require an appropriate radiation source and highly standardized conditions during radiation. This work reports on the design of a standardized irradiation device using a commercially available X-ray tube with a custom constructed lead collimator with two circular apertures and an animal bed plate, permitting synchronous irradiation of two animals. Dosimetry and the corresponding methodology for radiotherapy of human non-small cell lung cancer xenograft tumours transplanted to and growing subcutaneously on the right lower limb in a nude rat model were investigated. Procedures and results described herein prove the feasibility of use of the device, which is applicable for any investigation involving irradiation of non-tumorous and tumorous lesions in small animals.

[Tokalov S V, Enghardt W and Abolmaali N 2010 Tumour bed irradiation of human tumour xenografts in a nude rat model using a common X-ray tube; *J. Biosci.* **35** 203–207] **DOI** 10.1007/s12038-010-0024-4

1. Introduction

Reliable human tumour xenograft models in rodents are a vital part of preclinical cancer therapy investigations (Wolf and Abolmaali 2009). Animal experiments are thought to be more relevant for translational research than *in vitro* studies (Baumann *et al.* 2009). For example, tumours growing in irradiated tissue in rodents are frequently being used as experimental models of recurrent tumours in humans. Most of them show a prolonged period of latency and a reduced growth rate in comparison with tumours transplanted into intact tissue (Baumann *et al.* 1994). This effect was discovered at the beginning of the last century (Frankl and Kimball 1914) and was named later as the tumour bed effect (Stenstrom *et al.* 1955). The tumour bed effect has been mainly attributed to impaired neovascularization (Haveman *et al.* 2007). While the tumour bed effect has been intensively investigated, the design of tools for tumour bed irradiation (TBI) has received considerably less attention.

The long-standing absence of dedicated systems delivering local irradiation to small animal tumour models has led some investigators to design special radiation units (Deng *et al.* 2007; Rodriguez and Jeraj 2008; Matinfar *et al*. 2009). Others modified existing experimental (Graves *et al.* 2007) or clinical (Medina *et al.* 2008) devices that are usually not designed to deliver conformal doses to a target volume in small animals. This work is devoted to the development of standardized conditions for TBI using a commercially available X-ray device with dedicated collimators and an appropriate animal bed plate. The relevant dosimetry and the corresponding methodology for reproducible radiotherapy of human tumour xenografts in a nude rat model are described.

2. Materials and methods

2.1 *Radiation source and conditions*

Radiation exposure in our study was carried out by means of the commercially available X-ray YXLON MG325 device (200 kV, 20 mA, Yxlon International X-Ray GmbH, Hamburg, Germany, figure 1A), which was equipped with an animal positioning stage containing a collimator holder (figure 1B). The construction of the animal positioning stage allows a manual change of both the collimator plate and the corresponding animal bed plate before irradiation.

Keywords. Human tumour xenografts; radiation; rats; tumour bed

Whole body irradiation (WBI) with a dose of 4 Gy (dose rate: 0.877 Gy/min) for immunosuppression of animals delivered 2 days before tumour transplantation was carried out using a standard WBI lead collimator plate (thickness 12 mm, 220 x 180 mm square aperture, 0.5 mm Cu filter) and an animal WBI bed plate as described earlier (Tokalov *et al.* 2009).

To perform TBI with a dose of 15 Gy (dose rate: 0.877 Gy/min) which exclusively covers the target volume, a lead collimator (12 mm thick) with two circular apertures 2.0 cm in diameter (0.5 mm Cu filter, figure 1C) and an appropriate animal TBI plate (figure 1D) were custom made. The design of the collimator plate for TBI allowed irradiation of 2 circular areas and protection of other areas. This collimator plate for TBI permitted the placement of two anaesthetized rats, by fixating their right lower limbs and performing irradiation in the appropriate positions (figure 2A). The added filtering consisting of a 0.5 mm thick Cu filter was chosen for beam hardening, reducing the influence of the lower energy photons.

2.2 *Dosimetry*

Dosimetry was performed with the clinical dosimeter UNIDOS equipped with the Semiflex ionization chamber (PTW-Freiburg, Germany) using a set of water equivalent plate ($1 \times 5 \times 5$ cm^3) phantoms (PTW RW3 Slab Phantom 29672, Siemens, Germany). Due to the fitness of the phantoms used for the dosimetry, the irradiation dose was estimated at a depth of 5 mm (middle of the water equivalent plate).

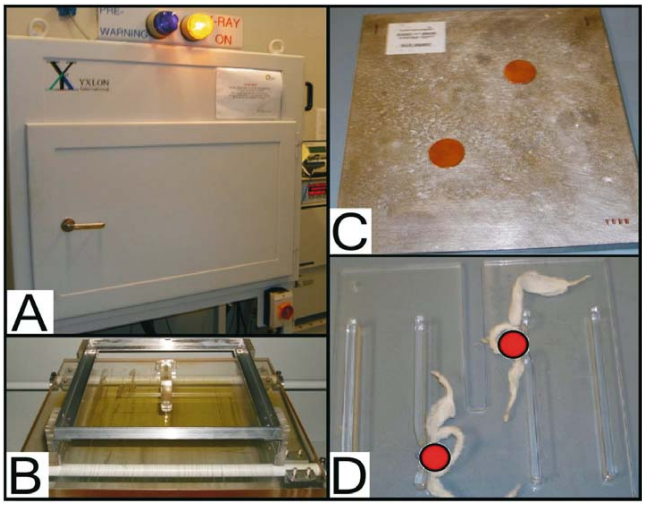

Figure 1. Radiation system. Commercially available X-ray system (**A**) with an animal positioning device (**B**) containing a collimator holder. The 12 mm thick lead collimator for tumour bed irradiation with two circular apertures 2.0 cm in diameter and 0.5 mm thick Cu filter (**C**) and the animal bed plate (**D**) are shown. Areas of the animal bed plate that underwent irradiation are marked in red.

Two-dimensional (2D) dose distribution was visualized using near tissue equivalent self-developing GAFCHROMIC EBT films (International Specialty Products, NJ, USA). For EBT film 2D dosimetry, a standardized protocol described earlier (Devic *et al.* 2007) was used. To reduce the effects of temperature-dependent fading and readout, storage, irradiation and readout were performed in the same environment. Additionally, the films were kept together to avoid differences in thermal histories. A delay of at least 24 h after the irradiation was maintained to allow the EBT films to self-develop before scanning. EBT films were scanned using an EPSON flatbed scanner (EPSON 4990, Seiko Epson Corporation, Japan) in the transmission mode. The red channel was extracted for analysis using a narrow band pass filter because EBT has a maximum response to red light at 633 nm (Fuss *et al.* 2007).

EBT films were analysed at a 10-fold magnification using the light channel of a Zeiss Axioplan-2 fluorescence microscope with a digital camera and corresponding software (AxioCam, Carl Zeiss, Germany).

The dose depth profile within the water-equivalent phantom was computed using the GEANT 4 Monte Carlo toolkit simulating the passage of X-ray photons through matter. The simulation results were compared with the measured dose values.

2.3 *Animals*

Male athymic 5-week-old nude rats (Harlan Laboratories GmbH, Borchen-Alfen, Germany) were kept five rats per cage with water and food *ad libitum* at least one week before the experiments. Animal housing and experiments were approved by the local animal care committee according to the institutional guidelines and the national animal welfare regulations. Intraperitoneal anaesthesia was performed with a ketamin 500 (120 mg/kg, Curamed, Germany)/xylazin (16 mg/kg, Rompun, Germany) mixture. Two days after irradiation (WBI or WBI+TBI), human non-small cell lung cancer cells (A549, DSMZ, Braunschweig, Germany) were transplanted by subcutaneous injection (5 x 10^6 cells in 0.1 ml of phosphate buffer saline) into the right lower limb in the middle of the area that had received TBI. Additionally, tumour-bearing animals with subcutaneously growing tumours in the right lower limb were placed in the animal bed made for the TBI (figure 2B) and irradiated using the respective collimator plates.

2.4 *Statistics*

The experimental results are expressed as the mean ± standard deviation of several independent experiments. Analysis of variance (ANOVA) was performed.

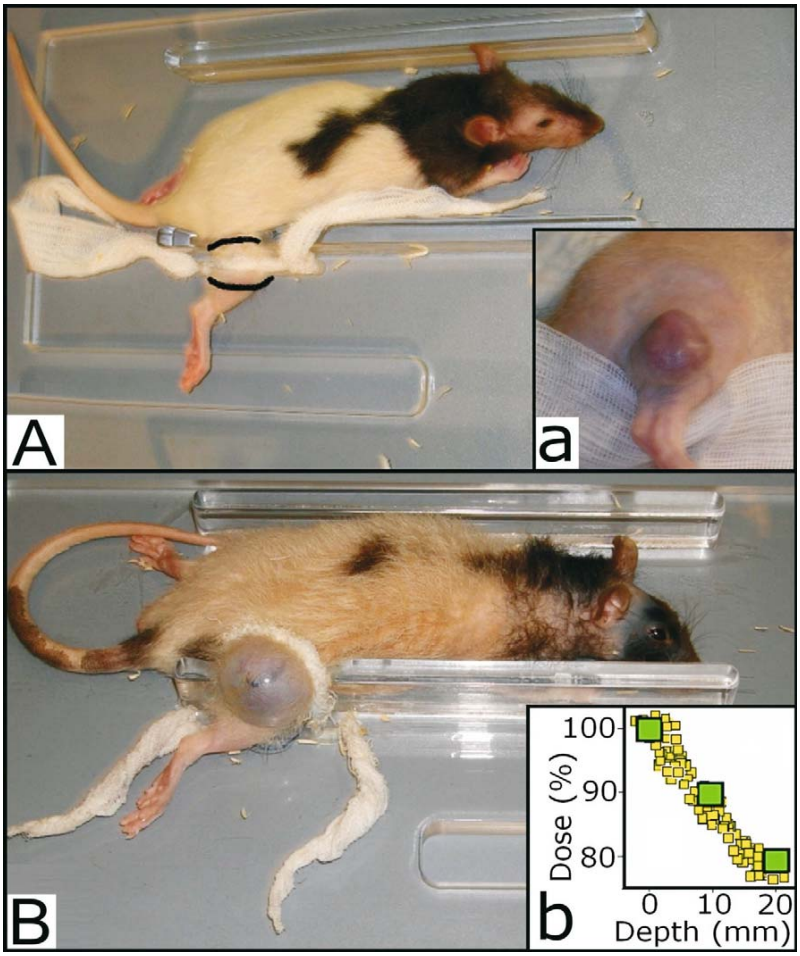

Figure 2. Tumour bed irradiation and radiotherapy. Positioning of a rat on the animal bed during tumour bed irradiation is illustrated (**A**). The irradiated area is delineated by black lines. A nodule with a volume of approximately 1000 mm^3 that grew (*inset* **a**) in the middle of the hairless irradiated area is shown. Positioning of a tumour-bearing rat on the animal bed during radiotherapy using the collimator for tumour bed irradiation is shown (**B**). Dose distribution along the depth of a 2 cm water equivalent phantom for Monte Carlo simulation of the X-ray photons passage through matter (yellow) and dose evaluation using a set of water equivalent plate phantoms (green) are presented (*inset* **b**). The curves have been normalized to 100% on the surface of phantoms.

3. Results

3.1 *Evaluation of the dose distribution with EBT lms*

Visual examination of the irradiated EBT films of the animal bed plate made for TBI showed two different areas (figure 3A). A dark blue round area (20 mm diameter) was clearly seen in the region of the EBT film that underwent TBI when the TBI collimator was used. The other parts of the EBT film were light blue in colour.

Examination of the irradiated EBT films by microscopy indicated highly localized dose deposition (figure 3B). The width of the edge between the areas where the colour of the EBT film changed from light to dark blue did not exceed 1 mm.

Direct dosimetry performed with the UNIDOS dosimeter revealed that after TBI the accumulated dose in the centre of the dark blue area was 15 Gy, while outside this area it did not exceed 7 cGy (figure 3B).

The analysis of 2D dose distributions in the selected areas using the EBT film/EPSON4990 atbed scanning system (figure 3C) revealed close agreement with visual examination.

Analysis of dose profiles through the centres of the irradiated areas (along the red line on figure 3C) confirmed the visual interpretations (figure 3D). A similar high level of accumulated dose with an inhomogeneity of less than 5% was revealed in the field which underwent TBI, and a quantitatively insignificant level of accumulated dose was recorded in the surrounding area protected from TBI.

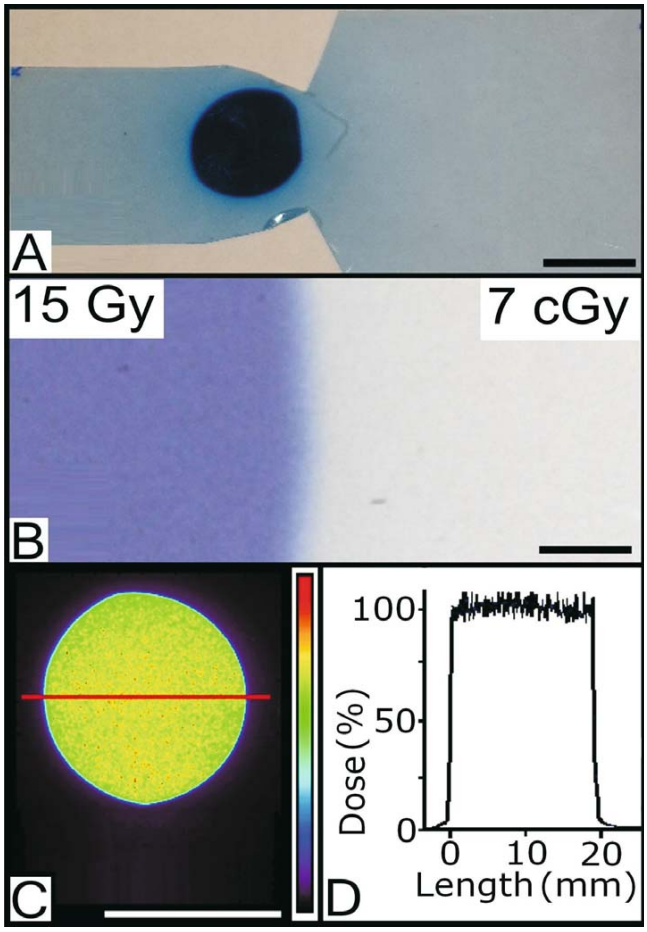

Figure 3. EBT film evaluation of the dose distribution. Visual (**A**), microscopic (**B**) and scanner (**C, D**) analysis of EBT films after tumour bed irradiation (15 Gy) is shown. Irradiated (dark blue) and protected (light blue) areas are clearly separated (**A**). A step gradient between both areas is seen (**B**). Doses in irradiated (15 Gy) and protected (7 cGy) areas after TBI is given (**B**). 2D dose distribution is reconstructed (**C**) and data profile across the irradiated area along the red line is shown (**D**). Bars equal 2 cm (**A**, **C**) and 2 mm (**B**).

3.2 *Experiments with animals*

At first, the established tool was applied for TBI of rats. To improve tumour take rates, animals were irradiated with WBI. Then they additionally underwent TBI and the corresponding areas were marked on the skin of animals (figure 2A). Subcutaneous injection of A549 cells in the centre of this area two days after irradiation induced tumour growth in the middle of the hairless irradiated area in the lower limb of nude rats (figure 2A, inset a).

In the second part of the work, the established tool was applied for radiotherapy of tumour xenografts (figure 2B). To evaluate the dose depth profile within the tumours, the dose depth profile within the water equivalent phantom was computed using the GEANT 4 Monte Carlo toolkit, simulating the passage of X-ray photons through matter. The result of this simulation was compared with the measured dose depth values using a set of water equivalent plate (1 x 5 x 5 cm^3) phantoms. Good agreement in the quantification of the dose depth value was found between the simulation and measurements for TBI of tumours in the lower limb of rats (figure 2, inset b). The dose decreased exponentially from its maximum value (set to 100%) at the top to ~80% at the bottom of the phantom. Therefore, dose inhomogeneities in tumours less than 2 cm in diameter did not exceed 10% during commonly applied radiotherapy (local tumour irradiation with 2 Gy) using TBI tools. The additive dose of TBI to WBI did not exceed 0.5 cGy.

4. Discussion

This work was devoted to the development of conditions for TBI and local tumour irradiation using a commercially available X-ray device. To this end, both the lead collimator and the animal bed plate for TBI were made. EBT films were employed to verify the 2D dose distribution in the plane of the rat bed plate. The high spatial resolution of EBT films makes them ideal for such purposes (Chiu-Tsao *et al.* 2005) because of their energy independence and high sensitivity (Deng *et al.* 2007). Using EBT films we were able to show that the thickness of the constructed TBI collimator was adequate to guarantee minimal penetration and dispersion of the X-ray beam outside the collimator's aperture. Administration of irradiation at a depth of 5 mm additionally improved dose delivery into the field of the tumour bed. As a result, the collimator permits TBI while protecting the rest of the body.

The new equipment allowed us to investigate tumour bed effects using human tumour xenografts in a rat model. Preliminary WBI has been used as the classic method for experimentally inducing immunosuppression in rodents and improve tumour take rates of human tumour xenografts in nude mice (Zips *et al.* 2009) and nude rats (March *et al.* 2001; Tokalov *et al.* 2008, 2009). It was found that after TBI (15 Gy), tumour nodules showed a prolonged latency period and a reduced growth rate in comparison with the control tumour-bearing rats (Tokalov *et al.* 2008, 2009).

It is well known that inhomogeneous radiation dose distributions in tumour may result in an incomplete elimination of tumour cells, which may lead to an incomplete therapeutic response to radiotherapy. The results of the phantom examination for the thickness of tumours less than 20 mm in diameter presented here showed that the difference between the entrance and exit dose did not reach 20%. This suggests that the delivered dose inhomogeneities over the corresponding target volume did not exceed ±10%

of the mean value. It needs to be noted that subcutaneous tumours are usually in the form of flat spheroids, at least at the beginning of their growth. As a result, the thickness of 20 mm diameter tumours in our study typically did not exceed even 10 mm.

The results of this work demonstrate that small modifications of commercially available X-ray devices allow us to perform radiotherapy of tumours subcutaneously transplanted into the right lower limb of rats with an accuracy similar to that obtained by using a clinical X-ray unit as published earlier (Medina *et al.* 2008). Obviously, a series of collimator plates for TBI with different apertures might be more suitable for radiotherapy investigations of tumours of different diameter.

The results and procedures described in the present study have shown the usefulness of a commercially available X-ray device equipped with a TBI collimator and an appropriate animal bed plate. This equipment was used for TBI and radiotherapy of non-small cell lung cancer tumours subcutaneously transplanted into the right lower limb of rats. The technique of local irradiation may be useful for investigations involving other types of human tumours in a nude rat model.

Acknowledgements

The authors wish to thank Arian Khaless and Christian Richter for providing departmental support. This research and the authors are supported by the Federal Ministry of Education and Research, Germany, BMBF Contract 03ZIK042.

References

Baumann M, Würschmidt F, Twardy A and Beck-Bornholdt H P 1994 Impact of tumor stroma on expression of the tumor bed effect in R1H rat rhabdomyosarcoma; *Radiat. Res*. **140** 432–436

Baumann M, Zips D and Appold S 2009 Radiotherapy of lung cancer: technology meets biology meets multidisciplinarity; *Radiother. Oncol*. **91** 279–281

Chiu-Tsao S T, Ho Y, Shankar R, Wang L and Harrison L B 2005 Energy dependence of response of new high sensitivity radiochromic films for megavoltage and kilovoltage radiation energies; *Med. Phys*. **32** 3350–3354

Deng H, Kennedy C W, Armour E, Tryggestad E, Ford E, McNutt T, Jiang L and Wong J 2007 The small-animal radiation research platform (SARRP): dosimetry of a focused lens system; *Phys. Med. Biol*. **52** 2729–2740

Devic S, Tomic N, Pang Z, Seuntjens J, Podgorsak E and Soares C 2007 Absorption spectroscopy of EBT model GAFCHROMIC film; *Med. Phys*. **34** 112–118

Frankl O and Kimball C P 1914 Uber die Beeinflüssung von Mäuse Tumoren durch Roentgenstrahlen; *Wien Klein. Woschr*. **27** 1448–1450

Fuss M, Sturtewagen E, De Wagter C and Dietmar G 2007 Dosimetric characterization of GAFCHROMIC EBT film and its implication on film dosimetry quality assurance; *Phys. Med. Biol*. **52** 4211–4225

Graves E, Chatterjiee R, Keall P, Gambhir S, Contag C and Boyer A 2007 Design and evaluation of a variable aperture collimator for conformal radiotherapy of small animals using a microCT scanner; *Med. Phys*. **34** 4359–4367

Haveman J, Rodermond H, van Bree C, Wondergem J and Franken N A P 2007 Residual late radiation damage in mouse stromal tissue assessed by the tumor bed effect; *J. Radiat. Res*. **48** 107–112

March T H, Marron-Terada P G and Belinsky S A 2001 Refinement of an orthotopic lung cancer model in the nude rat; *Vet. Pathol*. **38** 483–490

Matinfar M, Ford E, Iordachita I, Wong J and Kazanzides P 2009 Image-guided small animal radiation research platform: calibration of treatment beam alignment; *Phys. Med. Biol.* **54** 891–905

Medina L A, Herrera-Penilla B I, Castro-Morales M A, García-López P, Jurado R, Pérez-Cárdenas E, Chanona-Vilchis J and Brandan M E 2008 Use of an orthovoltage X-ray treatment unit as a radiation research system in a small-animal cancer model; *J Exp. Clin. Cancer Res.* **27** 57

Rodriguez M and Jeraj R 2008 Design of a radiation facility for very small specimens used in radiobiology studies; *Phys. Med. Biol*. **53** 2953–2970

Stenstrom K W, Vermund H, Mosser D G and Marvin J F 1955 Effects of roentgen irradiation on the tumor bed. I. The inhibiting action of local pretransplantation roentgen irradiation (1500 r alpha) on the growth of mouse mammary carcinoma; *Radiat. Res*. **2** 180–191

Tokalov S V, Schindler S, Abramyk A M and Abolmaali N D 2008 Establishment of NSCLC xenografts with different vasculogenesis; *Cellular Oncol*. **30** 135–136

Tokalov S, Glauert A, Mirus M, Prochnow W, Koch A, Abramyuk A, Wolf G, Baumann M *et al*. 2009 Vascularization in two different tumor xenograft models; *Eur. J. Cell Biol*. **88** 10–11

Wolf G and Abolmaali N 2009 Imaging tumour-bearing animals using clinical scanners; *Int. J. Radiat. Biol*. **85** 752–762

Zips D, Le K, Yaromina A, Dörfler A, Eicheler W, Zhou X, Geyer P, Hilberg F, *et al*. 2009 Triple angiokinase inhibition, tumour hypoxia and radiation response of FaDu human squamous cell carcinomas; *Radiother. Oncol*. **92** 405–410

MS received 3 February 2010; accepted 22 April 2010

*e*Publication: 6 May 2010

Corresponding editor: Rita Mulherkar