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Radiation Dose Uncertainty and Correction for a Mouse Orthotopic and Xenograft Irradiation Model

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Abstract

Purpose—In animal irradiation models, reported dose can vary significantly from the actual doses delivered. We describe an effective method for *in vivo* dose verification.

Materials and Methods—Mice bearing commercially-available cell line or patient-derived tumor cell orthotopic or flank xenografts were irradiated using a 160 kVp, 25 mA X-ray source. Entrance dose was evaluated using optically-stimulated luminescence dosimeters (OSLD) and exit dose was assessed using radiochromic film dosimetry.

Results—Tumor position within the irradiation field was validated using external fiducial markers. The average entrance dose in orthotopic tumors from 10 OSLDs placed on 2 different animal irradiation days was 514 ± 37 cGy (range: 437–545). Exit dose measurements taken from 7 radiochromic films on two separate days were 341 ± 21 cGy (a 34% attenuation). Flank tumor irradiation doses measured by OSLD were 368 ± 9 cGy compared to exit doses of 330 cGy measured by radiochromic film.

Conclusion—Variations related to the irradiation model can lead to significant under or overdosing *in vivo* which can affect tumor control and/or biologic endpoints that are dose dependent. We recommend that dose measurements be determined empirically based on the mouse model and irradiator used and dose compensation adjustments performed to ensure correct and appropriate doses.

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Declaration of Interest

The authors report no declarations of interest

Keywords

Radiation dosimetry; radiophysics; mouse tumor xenografting

Introduction

The use of radiation therapy remains a cornerstone therapy for various human malignancies. Therefore, biologic research routinely employs cell-based, tissue and small animal irradiation models to try and better understand molecular and cancer biology. These experiments have been traditionally performed using gamma irradiators utilizing Cs-137 or Co-60. More recent tissue and small animal irradiators utilize x-ray tubes to generate high energy x-rays (equivalent to gamma rays) rather than rely on radioactive source for modern radiobiologic research. Both flank and orthotopic xenograft tumor models are routinely used in radiation-based animal experiments and the recent emergence of patient derived xenograft (PDX) models allow more sophisticated experimentation to understand human tumor biology and accelerate drug discovery (Kimple et al., 2013, Keysar et al., 2013b, Keysar et al., 2013a, Gan et al., 2014). Therefore accurate ways to measure delivered dose to tumors both *in vitro* and *in vivo* are necessary.

Although, dose calculations for megavoltage photon beams evolved significantly in the past decade, more progress is needed for accurate dose calculations in the 120–300kVp energy range. With the introduction of image guided small animal irradiation platforms (Grant et al., 2014, Wong et al., 2008, Zhou et al., 2010), CT image based dose calculations for small animal have been implemented where dose calculations are performed using Monte Carlo methods. However, such platforms are not widely available in small animal irradiation facilities as many researchers utilize conventional X-ray or gamma ray irradiators. In conventional irradiators, simplified animal models use dose calculations relying on point dose measurements in the irradiation chamber and factors affecting accurate dose estimation, such as tissue thickness and composition or the effects of scattered radiation, are often approximated or omitted.

To better understand the discrepancy between intended and delivered dose to target, various tools are available for *in vivo* dose verification. OSLD chips are suitable to place directly on animals, and can provide accurate animal and tissue dosimetry (Lu et al., 2013). OSLD systems may not be available in small animal irradiation facilities, however, access to this technology can be obtained through collaborations with Radiation Oncology departments. A fast and reliable alternative would be the use of radiochromic films which allow for high spatial resolution and accurate dose measurement and can be read out using a flat-bed document scanner.

In this study, we employed both OSLD and radiochromic film to determine entrance and exit doses in both head-and-neck orthotopic and traditional xenograft flank tumor irradiation animal models. Our results demonstrate that radiation overdosing or under-dosing occurs, and should be accounted for when designing animal, tissue or cell based experiments.

Materials and Methods

Animal Irradiation Apparatus and Radiation Dosimetry

A commercial animal restrainer (experimental conditioning unit) was modified through Braintree Scientific, Inc (Braintree, MA) to fit on a custom plexiglass baseboard (Figure 1A). An institutional animal review committee independently reviewed and approved all protocols for animal experimentation prior to initiation of this project. Animals were anesthetized using 60 mg/kg ketamine and 8 mg/kg xylazine. Animals with orthotopic tumors measuring 75–100 mm³ were deemed appropriate for analysis and anesthetized animals were placed within the conditioning unit. Representative mouse head-and-neck tumors are circled in red (Figure 1B, top figure). A radio-opaque BB was placed at the base of the mouse's floor-of-mouth tumor to demarcate the most inferior portion of the tumor, and the animal was carefully placed within the animal restrainer on the ventral-stomach surface. In addition, attempts were made to position them on their backs initially as irradiation of the mouse head and tumor in the floor of mouth would have been easier. However, given their pliable bodies would not remain in a fixed position, this was setup was abandoned. The choice to place the animal on their stomach as opposed to their back was chosen for reproducibility and consistency as this is the most routine and commonly used method by experimentalists. OSLDs (nanoDot™, Landauer, Chicago, IL) were placed on the top of the head restraining device to measure radiation entry dose. Radiochromic film (Gafchromic EBT2, ISP, Wayne, NJ) was placed underneath the animal restrainer prior to irradiation in order to measure radiation exit dose. Individual restrainers were then bolted on to the custom baseboard with their heads all pointing towards the middle (Figure 1C).

A custom cerrobend (Lipowitz alloy) shield was engineered due to its ease of use and common employment for custom radiation treatment blocks. It is composed from elemental bismuth (50%), lead (26.7%), tin (13.3%) and cadmium (10%). This 7 mm thick, 32 x 32 cm² cerrobend shield with a 10 cm in diameter circular cutout in the middle of the shield was placed on top of pylons mounted to the baseboard with the cutout exposing only the mouse head/neck area (denoted by the black head piece) (Figure 1D). On average, mouse head and neck separation from top of cranium to bottom of jaw-tumor was about 1.25 – 1.5 cm depending on mouse weight. Animal head-and-neck tumors were irradiated to a total dose of 1500 cGy: 500 cGy delivered once per week for a total of 3 weeks using the RS-2000 (Rad Source, Suwanee, GA) small animal irradiator. The device utilizes a fixed anode X-ray tube operating at 160 kV voltage and 25 mA current. Seven animals were irradiated.

The absolute dose rate of the small animal irradiator was measured using a farmer type ion chamber and AAPM TG-61 protocol (Ma et al., 2001) at the calibration point, which was defined as the central axis of the base surface in the irradiation chamber. In order to develop a fast method to verify delivered dose, we chose to use radiochromic film because of its ease of use, deformability that it would create a permanent (film) record of treatment and generating a dose-to-exposure standard curve is reasonably straight forward process. Calibration of OSLDs and optical density to dose calibration for radiochromic films were generated up to 1000 cGy using vendor (Yahnke, 2009) and AAPM recommended protocols

(Niroomand-Rad et al., 1998). In our study, the OSLD's used had an inherent dosimetric accuracy of plus-minus 5% which was independent of energy or backscatter. Furthermore, their dosimetric sensitivity deteriorate with total accumulated dose. Absolute dose measurements and calibration of farmer-type ion chamber, OSLD and radiochromic films were all performed at the same day to assure identical calibration conditions. RS-2000 irradiator utilizes a boron carbide frame to increase dose uniformity. This frame was removed during both dose calibrations and animal experiments to be able to accommodate the animal restraining device and cerrobend radiation shield. Calibration and readout of OSLDs were performed using MicroStar InLight Reader (Landauer, Chicago, IL). Radiochromic films were digitized using a 16-bit film digitizer ((Dosimetry Pro Advantage (Red), Vidar, Herndon, VA), and AAPM TG 55 (Niroomand-Rad et al., 1998) protocol was used to convert optical density (OD) to dose. RIT113 film analysis software (Radiological Imaging Technology, Colorado Springs, CO) was used to control film digitizer and convert OD to dose. The analysis of films was performed using in-house developed Matlab scripts (MathWorks, Natick, MA). As the color development of radiochromic films continue after irradiation, films were digitized at least 24 hours after irradiation.

A similar custom, in-house flank irradiation apparatus (Figure 2A) was designed from cerrobend to measure flank irradiation using the principles already described. BBs were placed on the mouse flank to identify location of flank tumor in radiochromic films (Figure 2B). In order to prevent unnecessary mouse tail irradiation, the mouse tail was carefully taped on the mouse's back so that it was well out of the irradiation field. The anesthetized mouse was placed prone on top of radiochromic film and the flank irradiation device was placed on top of the mouse making sure that the entire body was well underneath the shield. The mouse and shield were then subjected to 300 cGy irradiation (Figure 2C) twice-a-week for a total of two weeks. A representative mouse is shown post-irradiation under the device with the radiochromic film underneath demonstrating the area that was shielded (yellow) and the area irradiated (dark green) (Figure 2D).

Results

Absolute dose rate (dose to water) was 112 cGy/minute at the calibration point. OSLDs were used to measure entrance dose for the head neck model. A dose of 500 cGy was prescribed for the orthotopic tumors. Eleven different total measurements were performed on 7 different animals on two different days. Three animals did not get a second measurement because of three of the OSLDs experienced readout issues. The mean entrance dose was 514 ± 37 cGy (range 437 – 545) (Table I). Exit dose was measured on two separate days for 7 animals (Table II). Seven animals had a radio-opaque BB placed at the inferior most portion of the tumor validating the tumor was within the radiation treatment portal. To measure exit dose, radiochromic film was placed underneath the animal irradiation device prior to irradiation and measured on two separate days. Location of BB within the radiation field was evidence that the tumor was within the radiation treatment portal (Figure 3A).

A cumulative dose histogram (CDH), was generated (Figure 3B) for the region of interest (ROI) within the red square (Figure 3C). While 98% of the ROI gets at least 300 cGy, only 21 % of the ROI gets 400 cGy or higher. The CDH is useful to demonstrate the large exit

dose variations in the mouse head, however it does not reflect the exit dose specifically at the tumor location. To better estimate tumor exit dose, dose was measured in four smaller ROIs adjacent to the BBs that marked tumor locations (Figure 3D). Each ROI had an area of $1.5 \times 1.5 \text{ mm}^2$, and they were placed on the top, bottom, left, and right side of each BB. The exit dose at orthotopic tumor location was calculated by averaging the dose from 4 ROIs (Figure 3B). The average exit dose measured from all 7 animals using this approach was $341 \pm 21 \text{ cGy}$. The difference between measured entrance dose versus exit dose corresponds to a 34% decrease in the animal prescription dose.

We similarly measured the entrance dose and exit dose to the bilateral mouse flanks using OSLD and radiochromic film measurements (Table I) OSLDs were placed on top of the mouse flank tumor. A dose of 300 cGy was prescribed to the bilateral mouse flanks. The average measured entrance dose was $368 \pm 9 \text{ cGy}$. The average measured exit dose by radiochromic film was $330 \pm 12 \text{ cGy}$. The use of radiochromic film demonstrated that the mouse flank and tumor were adequately exposed underneath the flank irradiation shield (Figure 2E). A representative radiation isodose plot is shown detailing the predicted doses delivered to the mouse flank and tumor with the greatest doses at the flank edge compared towards midline where the dose falls off (Figure 2F). The exit dose underneath the radiation shield was negligible. The difference between measured entrance and exit dose is approximately 12%.

Discussion

Radiation is routinely used for both *in vitro* and *in vivo* experiments in oncology to study a variety of different cancer processes. There is a need for accurate models in order to try and recapitulate the human system. The emergence of PDX models which utilize orthotopic implantation therefore play an important role particularly if radiation treatment is to be used. A variety of small animal irradiators utilizing x-ray tubes operating in the 120–300kVp energy range are commercially available. With respect to traditional gamma irradiators, these irradiators allow more conformal radiation delivery, since the radiation shields, or apertures, can be easily formed from relatively thin sheets of lead or cerrobend. The dose fall-off is also sharper at the edge of the aperture due to small size of x-ray focal spot. Our paper is one of the first to describe a fast and simple method for measuring radiation dose delivered for either a mouse orthotopic head-and-neck or flank tumor model which can be easily employed to verify dose at the time of treatment.

When commissioning any irradiator, a robust and dependable method is necessary to validate absolute dose output, but additional methods are needed to verify accurate dose delivery to either cells or tumor target. The use of radiochromic film has inherent advantages including a large dynamic radiation dose range for measurement, higher spatial resolution, ease of measurement not requiring chemical film development like traditional silver halide film, and low upfront and general material costs. Furthermore, the use of radiochromic film allows for permanent records that demonstrate the region irradiated compared to areas that were not irradiated as demonstrated in this paper.

One drawback with the use of film in this model is that it [film] cannot be cut down to small sizes amenable for attachment to a mouse as it cannot be read by the film reader nor can they be made to conform to the mouse's body. OSLD's, in comparison do not provide spatial variations in dose, but they can be directly placed on the target due to their small size which is a significant advantage which explains their widespread use for *in vivo* medical dosimetry. Unlike radiochromic films, they can be read within minutes after irradiation allowing for rapid evaluation of delivered dose within hours after treatment.

Our results demonstrate that dose calibration measurements in the irradiation chamber may not be an accurate surrogate for the dose delivered to the tumor. By using a head/neck orthotopic tumor model, we demonstrated that exit doses can indicate as much as 34% underdose compared to a flank tumor model which can experience a 12% overdose. Knowing this information can allow a researcher to adjust radiation doses accordingly for improved accuracy and precision if needed. We acknowledge that our system cannot accurately approximate doses if the tumor is within the mouse (ie. orthotopic prostate or pancreas models) compared to superficial lesions such as the flank and orthotopic head and neck models. However, by measuring the entrance and exit doses, one can get an approximation of the dose within the animal. One way to more accurately measure dose delivered to an internal tumor, a small animal CT scanner and a radiation treatment planning system would be necessary to approximate tumor dose.

A variety of factors can explain the differences in measured doses and calibration dose (Khan, 2010). First, X-ray energy plays a role in attenuation. The 160 kVp x-rays are more easily attenuated by bone due to the photo-electric effect. In comparison, photoelectric effect is less pronounced at higher energies and has improved penetration through bone. Therefore, exit dose would more likely reflect the prescribed dose. Another issue that may attribute to dose discrepancy is shorter source-to-target distance. For the flank tumors we studied, the animals were placed on a raised platform to hold the flank irradiation shield; this brought the animal closer toward the radiation source and in turn increased the dose to the tumor. This change in position increased the dose that we observed and can be partially explained by the inverse square law. The distance between the x-ray source and the bottom surface of the irradiation tray is 48.6 cm. If the mouse is placed 2 cm above the bottom surface of the irradiation chamber the dose rate would go up by 9%. In 100 to 200 keV energy range, scattered x-rays also play a major role in observed differences between measured and targeted dose levels. The placement of the radiation shield, the size of the irradiated area, and the location of the target in relation to other objects that cause scatter may not be accounted by dosimetric characterization of the irradiation chamber only. For example, the contribution of backscattered radiation from the bottom surface of the irradiation chamber to dose at tumor location is a function of irradiated area, beam quality, source-to-surface distance, and distance to bottom surface. The the variation in backscatter dose can have a large impact on variation in measured dose (Ma et al., 2001). Thus, measurement of dose during irradiation experiments can quantify the cumulative effect of various dose altering sources, and it can allow for more accurate *in vivo* or *in vitro* radiation dose delivery to tumor or tissue being studied.

One should also note that the dosimetric response of radiochromic films and OSLDs may also introduce errors to dose measurements. For example, dosimetric sensitivity of OSLD can vary more than a factor of two in the 125 kVp to 250kVp energy range. Also, its sensitivity exhibits a nonlinear dependence on the magnitude of delivered dose, the type of photon energy used (kilovoltage vs megavoltage), and type of particle used (electron, proton, carbon ion) (Reft, 2009). To account for energy-dependent sensitivity variations, the OSLDs were calibrated under reference conditions and within the dose range used during *in vivo* experiments, as described in the Materials and Methods section. Although our calibration approach reduced the energy and dose dependent uncertainties, it did not account for variations in beam energy due to attenuation and scattering (Al-Senan and Hatab, 2011). In contrast, the sensitivity of radiochromic film exhibits less dependence on energy and delivered dose (Lindsay et al., 2010), however their sensitivity may be dependent on how the film is handled or manufactured. Although the recommendations in reference (Niroomand-Rad et al., 1998) were followed in our experiments, we have not quantified the dosimetric errors introduced by our film-readout procedures.

To improve dosimetric accuracy in small animal irradiation experiments, new small animal radiation treatment planning software and novel animal irradiation platforms have recently become available (van Hoof et al., 2013, Verhaegen et al., 2011, Clarkson et al., 2011, Wong et al., 2008). Such treatment planning systems and irradiation platforms utilize Monte Carlo based dose calculations and CT image of the irradiated animal to improve the accuracy of delivered dose. However, a practical limitation to wide-spread use of this technology is that it is cost prohibitive and the number of facilities which would have this software is likely to be very limited.

For investigators planning small animal irradiation using a new or unfamiliar animal irradiator with access to a human or veterinary radiation oncology facility, we propose the following work flow to assist with dose determination. First, absolute dose rate is measured under reference conditions by using AAPM's TG 61 protocol. . Second, the *in vivo* dosimeter, i.e. radiochromic film or OSLD, is calibrated under the same irradiation conditions. Finally, for small animal (tumor) dose measurements, the dosimeter is placed on (in proximity to) the tumor-tissue and the dose is measured realizing the inherent limitations of the dosimeter. Using this method, one can obtain a close approximation of dose that the tissue or tumor is receiving, adjust the irradiator dose output, and thereby improve total dose approximation to the tumor for the "actual" *in vivo* experiment. We recommend measurement of dose on the first day of experimentation to account for differences between the planned and delivered dose. Finally, choice of the dosimeter (film versus OSLD) should be made on an empirical basis and the device adjusted to fit the mouse-irradiation circumstance. Whether dosimetric verification is needed with every treatment is at the discretion of the experimentalist, we do recommend establishing standard reproducible conditions which can be applied routinely to small animal irradiation in order to minimize dosimetric variation.

Conclusion

We have demonstrated a simple and fast means to determine radiation exit dose for an orthotopic head-and-neck and a xenograft flank tumor model using radiochromic film. Radiation dose to cells or tumor can be affected by a variety of different processes including source energy, distance from radiation source to target (i.e., position) and scattered radiation dose. These processes can lead to both over- and under-dosing and should be known so researchers can adjust accordingly. We recommend the described workflow to help with empirically determining the radiation dose for *in vivo* experimentation.

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References

- AL-SENAN RM, HATAB MR. Characteristics of an OSLD in the diagnostic energy range. *Med Phys.* 2011; 38:4396–405. [PubMed: 21859040]
- CLARKSON R, LINDSAY PE, ANSELL S, WILSON G, JELVEH S, HILL RP, JAFFRAY DA. Characterization of image quality and image-guidance performance of a preclinical microirradiator. *Med Phys.* 2011; 38:845–56. [PubMed: 21452722]
- GAN GN, EAGLES J, KEYSAR SB, WANG G, GLOGOWSKA MJ, ALTUNBAS C, ANDERSON RT, LE PN, MORTON JJ, FREDERICK B, RABEN D, WANG XJ, JIMENO A. Hedgehog signaling drives radioresistance and stroma-driven tumor repopulation in head and neck squamous cancers. *Cancer Res.* 2014
- GRANTON PV, DUBOIS L, VAN ELMPT W, VAN HOOFF SJ, LIEUWES NG, DE RUYSSCHER D, VERHAEGEN F. A longitudinal evaluation of partial lung irradiation in mice by using a dedicated image-guided small animal irradiator. *Int J Radiat Oncol Biol Phys.* 2014; 90:696–704. [PubMed: 25200196]
- KEYSAR SB, ASTLING DP, ANDERSON RT, VOGLER BW, BOWLES DW, MORTON JJ, PAYLOR JJ, GLOGOWSKA MJ, LE PN, EAGLES-SOUKUP JR, KAKO SL, TAKIMOTO SM, SEHRT DB, UMPIERREZ A, PITTMAN MA, MACFADDEN SM, HELBER RM, PETERSON S, HAUSMAN DF, SAID S, LEEM TH, GODDARD JA, ARCAROLI JJ, MESSERSMITH WA, ROBINSON WA, HIRSCH FR, VARELLA-GARCIA M, RABEN D, WANG XJ, SONG JI, TAN AC, JIMENO A. A patient tumor transplant model of squamous cell cancer identifies PI3K inhibitors as candidate therapeutics in defined molecular bins. *Mol Oncol.* 2013a; 7:776–90. [PubMed: 23607916]
- KEYSAR SB, LE PN, ANDERSON RT, MORTON JJ, BOWLES DW, PAYLOR JJ, VOGLER BW, THORBURN J, FERNANDEZ P, GLOGOWSKA MJ, TAKIMOTO SM, SEHRT DB, GAN GN, EAGLES-SOUKUP JR, SERRACINO H, HIRSCH FR, LUCIA MS, THORBURN A, SONG JI, WANG XJ, JIMENO A. Hedgehog signaling alters reliance on EGF receptor signaling and mediates anti-EGFR therapeutic resistance in head and neck cancer. *Cancer Res.* 2013b; 73:3381–92. [PubMed: 23576557]
- KHAN, FM., editor. *The Physics of Radiation Therapy.* 4. Philadelphia: Lippincott Williams & Wilkins; 2010.
- KIMPLE RJ, HARARI PM, TORRES AD, YANG RZ, SORIANO BJ, YU M, ARMSTRONG EA, BLITZER GC, SMITH MA, LORENZ LD, LEE D, YANG DT, MCCULLOCH TM, HARTIG GK, LAMBERT PF. Development and characterization of HPV-positive and HPV-negative head

and neck squamous cell carcinoma tumorgrafts. Clin Cancer Res. 2013; 19:855–64. [PubMed: 23251001]

LINDSAY P, RINK A, RUSCHIN M, JAFFRAY D. Investigation of energy dependence of EBT and EBT-2 gafchromic film. Med Phys. 2010; 37:571–6. [PubMed: 20229865]

LU L, BONDRA K, GUPTA N, SOMMERFELD J, CHRONOWSKI C, LEASURE J, SINGH M, PELLOSKE CE. Using NanoDot dosimetry to study the RS 2000 X-ray biological irradiator. Int J Radiat Biol. 2013; 89:1094–9. [PubMed: 23786571]

MA CM, COFFEY CW, DEWERD LA, LIU C, NATH R, SELTZER SM, SEUNTJENS JP. AAPM protocol for 40-300 kV x-ray beam dosimetry in radiotherapy and radiobiology. Med Phys. 2001; 28:868–93. [PubMed: 11439485]

NIROOMAND-RAD A, BLACKWELL CR, COURSEY BM, GALL KP, GALVIN JM, MCLAUGHLIN WL, MEIGOONI AS, NATH R, RODGERS JE, SOARES CG. Radiochromic film dosimetry: recommendations of AAPM Radiation Therapy Committee Task Group 55. American Association of Physicists in Medicine. Med Phys. 1998; 25:2093–115. [PubMed: 9829234]

REFT CS. The energy dependence and dose response of a commercial optically stimulated luminescent detector for kilovoltage photon, megavoltage photon, and electron, proton, and carbon beams. Med Phys. 2009; 36:1690–9. [PubMed: 19544786]

VAN HOOFF SJ, GRANTON PV, VERHAEGEN F. Development and validation of a treatment planning system for small animal radiotherapy: SmART-Plan. Radiother Oncol. 2013; 109:361–6. [PubMed: 24183860]

VERHAEGEN F, GRANTON P, TRYGGESTAD E. Small animal radiotherapy research platforms. Phys Med Biol. 2011; 56:R55–83. [PubMed: 21617291]

WONG J, ARMOUR E, KAZANZIDES P, IORDACHITA I, TRYGGESTAD E, DENG H, MATINFAR M, KENNEDY C, LIU Z, CHAN T, GRAY O, VERHAEGEN F, MCNUTT T, FORD E, DEWEESE TL. High-resolution, small animal radiation research platform with x-ray tomographic guidance capabilities. Int J Radiat Oncol Biol Phys. 2008; 71:1591–9. [PubMed: 18640502]

YAHNKE, C. Calibrating the microStar. Landauer; 2009.

ZHOU H, RODRIGUEZ M, VAN DEN HAAK F, NELSON G, JOGANI R, XU J, ZHU X, XIAN Y, TRAN PT, FELSHER DW, KEALL PJ, GRAVES EE. Development of a microcomputed tomography-based image-guided conformal radiotherapy system for small animals. Int J Radiat Oncol Biol Phys. 2010; 78:297–305. [PubMed: 20395069]

Figure 1. Custom rodent head and neck irradiation device

A. Five piece, custom small animal restraining device. **B.** Individual animal restraining device with anesthetized athymic nude mouse. Side profile (bottom) and under-side profile (top) shown. Red circle denotes orthotopic floor-of-the-mouth head-neck tumor. **C.** Animals placed on base plate in sets of 3–5 animals per irradiation cycle. **D** Custom 7 mm cerrobend shield affixed on top of animal restraints and only exposing mouse head-neck region.

Figure 2. Dosimetric evaluation of flank implanted xenograft tumors

A. Custom flank irradiation shield constructed in-house from cerrobend. **B.** Anesthetized non-SCID gamma mouse pre-irradiation. Tail has been fastened to its back and is laid out on top of the radiochromic film. **C.** Custom flank irradiation shield placed on top of animal exposing flanks. Animals were then irradiated with 300 cGy. **D.** Removal of flank irradiation shield demonstrating mouse alignment to irradiated film. Athymic nude mouse shown here for demonstration purposes. Film in yellow is non-irradiated while dark green denotes irradiated. **E.** Digitized film shows outline of mouse flank being irradiated (grey) and irradiation shield (black). **F.** Utilizing the digitized images, representative isodose lines corresponding to different exit doses were attributed. Higher exit doses being towards the lateral edges (370 cGy) compared to the edge of the shield (310 cGy).

Figure 3. Dosimetric evaluation of orthotopically implanted head-neck tumors

A. Animals were irradiated with a dose prescription of 500 cGy. Radiochromic film was sandwiched between the animal restraining device and the plexiglass base plate. An OSLD was placed on the apparatus surface to measure entrance dose and a radio-opaque BB (red arrow) was placed in the inferior most portion of the palpable tumor. **B and C.** Cumulative dose histograms were generated for each mouse-tumor based on the region of interest within the red square. tumor dose was primarily between 300 to 400 cGy. **D.** Four smaller regions of interest were identified around the BB and used for determining tumor exit dose for all 7 animals analyzed in these experiments.

Table I

Average Entrance Dose Measured by OSLD

Mouse Head Neck Apparatus	
Mean, Total (cGy)	514
SD, Total (cGy)	37
Range (cGy)	437 – 545
Mouse Flank Apparatus	
Mean, Total (cGy)	368
SD, Total (cGy)	9
Range (cGy)	355 – 377

cGy = centigray; SD = standard deviation

Table II
Average Radiochromic Film Based Exit Dose Measurements for the Mouse Head Neck Apparatus

Region of Interest (ROI)	M1	M2	M3	M4	M5	M6	M7
ROI1 (cGy)	411	318	317	325	461	347	291
ROI2 (cGy)	354	333	310	331	353	330	275
ROI3 (cGy)	420	329	316	369	417	325	297
ROI4 (cGy)	358	308	320	307	377	348	287
Mean dose (cGy)	386	322	316	333	402	338	341
SD (cGy)	35	11	4	26	47	12	21

ROI = region of interest; cGy = centigray; SD = standard deviation