Development of a PTV margin for preclinical irradiation of orthotopic pancreatic tumors derived from a well-known recipe for humans

Severin Kampfer [a,b,*], **Sophie Dobiasch** [a,c], **Stephanie E. Combs** [a,c,d], **Jan J. Wilkens** [a,b,c]

[a] Department of Radiation Oncology, School of Medicine and Klinikum rechts der Isar, Technical University of Munich (TUM), Munich, Germany
[b] Physics Department, Technical University of Munich (TUM), Garching, Germany
[c] Institute of Radiation Medicine (IRM), Department of Radiation Sciences (DRS), Helmholtz Zentrum München, Neuherberg, Germany
[d] German Cancer Consortium (DKTK), Partner Site Munich, Germany

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Abstract

In human radiotherapy a safety margin (PTV margin) is essential for successful irradiation and is usually part of clinical treatment planning. In preclinical radiotherapy research with small animals, most uncertainties and inaccuracies are present as well, but according to the literature a margin is used only scarcely. In addition, there is only little experience about the appropriate size of the margin, which should carefully be investigated and considered, since sparing of organs at risk or normal tissue is affected.

Here we estimate the needed margin for preclinical irradiation by adapting a well-known human margin recipe from van Herck et al. to the dimensions and requirements of the specimen on a small animal radiation research platform (SARRP). We adjusted the factors of the described formula to the specific challenges in an orthotopic pancreatic tumor mouse model to establish an appropriate margin concept. The SARRP was used with its image-guidance irradiation possibility for arc irradiation with a field size of 10 × 10 mm^2 for 5 fractions. Our goal was to irradiate the clinical target volume (CTV) of at least 90% of our mice with at least 95% of the prescribed dose. By carefully analyzing all relevant factors we gain a CTV to planning target volume (PTV) margin of 1.5 mm for our preclinical setup.

The stated safety margin is strongly dependent on the exact setting of the experiment and has to be adjusted for other experimental settings. The few stated values in literature correspond well to our result. Even if using margins in the preclinical setting might be an additional challenge, we think it is crucial to use them to produce reliable results and improve the efficacy of radiotherapy.

Keywords: CTV to PTV margin; Image-guided high-precision radiation; Pancreatic cancer; Preclinical tumor mouse model; Small-animal radiation research platform; Translational research

* Corresponding author: Severin Kampfer, Klinikum rechts der Isar, Klinik und Poliklinik für RadioOnkologie und Strahlentherapie, Ismaninger Straße 22, 81675 München, Germany.

E-mail addresses: Severin.Kampfer@gmx.de (S. Kampfer), Sophie.Dobiasch@tum.de (S. Dobiasch), StephanieElisabeth.Combs@mri.tum.de (S. E. Combs), Wilkens@tum.de (J. J. Wilkens).

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Introduction

In human radiation therapy, a safety margin is essential at least for a successful curative irradiation of tumors. In their report number 83, the International Commission on Radiation Units & Measurements (ICRU) [1] define several volumes related to radiation therapy, including GTV (gross tumor volume, which is the macroscopic tumor), CTV (clinical target volume: GTV plus microscopic spread), and PTV (planning target volume: CTV plus safety margin to account for uncertainties in the process of planning and delivery of radiation therapy). The GTV is typically contoured on a CT scan (partly with additional imaging), while the CTV is derived from the GTV and additional tumor knowledge. The PTV is a geometrical structure that is created from the CTV by adding a safety margin that depends mainly on the technical equipment and technique (for planning and delivery) as well as on the treated individual and the tumor location. The prescribed dose for the therapy is planned to the PTV, to ensure a minimum dose to the CTV is delivered, despite of typically occurring inaccuracies and uncertainties. In addition, the irradiated volume should be kept as small as possible to reduce side effects. In human radiotherapy, there are different possibilities to determine the appropriate safety margin, which is – amongst others - depending on motion of the tissue and uncertainties of the machine hardware. One very common and well-known recipe for human radiotherapy was published by van Herck et al. in 2000 [2]. They calculate the needed safety margin for defined conditions in radiotherapy based on several different factors. Even if the radiation equipment in the field has dramatically changed since the year 2000, particularly in terms of image guidance, a safety margin is still needed. Today, there is growing interest in even more sophisticated solutions like 'margin-of-the-day' strategies (cf. [3]).

In preclinical radiotherapy research with small animals, most uncertainties and inaccuracies are present as well and may in some extent be comparable to the ones in human therapy. That means a safety margin is needed for the same reasons as in human radiotherapy at least if the aim is to irradiate the whole CTV with at least the minimum dose and if a large field irradiation (much larger as the CTV is) should be avoided if possible. Nevertheless, only a few groups report about the creation and needed size of a PTV margin in small animals. The size of the margin should carefully be investigated and considered, since bigger margins lead to less sparing of organs at risk (OARs) or normal tissue [4] and the volume of a spherical margin increases with r^3 (r: radius). The bigger a PTV is, the more tissue will be irradiated. Amongst other challenges in small animal precision radiotherapy [5,6], like target motion [7], accuracy and precision of small field dosimetry, and methods to verify the dose distribution, the definition of a correct safety margin is still a major issue. This was also formulated by an ESTRO ACROP guideline earlier [6].

Up to now, there are no clear guidelines or rules how to create an appropriate margin in the context of the very different dimensions of the specimen. Also the reporting of margins is not standardized, some groups do report their used margins [8,9], some even explain how to determine the extent of the margin [8], or formulated a simple margin recipe for a moving lung tumor [10], and some do not mention whether a margin was used [11].

One possible approximation to the needed margin for the setup in preclinical research could be to implement and scale one of the existing recipes for human radiotherapy. In this work, we transfer the well-known margin recipe from van Herck et al. [2] to the dimensions and requirements of specimen on a small animal irradiation device. The translation of the more than 20 years old recipe is done in the awareness of some principle differences to the abilities today like the - also for preclinical methods available - (daily) image guidance and the availability of a higher conformity in treatment beams. In addition, there are possibilities to use arcs for therapy and do even more sophisticated methods to irradiate small animals, nearly comparable to the treatments in human radiotherapy. Nevertheless, most of research with small animal irradiation is still done with simple and less conformal techniques like single or two opposing beams or at least static beams and just rectangular or static circular fields. This may partly be comparable with the standards in human radiotherapy at the days when the margin recipe from van Herck et al. was created. In any case, if the target should be irradiated with the minimum dose, a certain safety margin is required to determine the needed minimum field size. We developed the needed margin for our experimental setup by adjusting the single factors from the recipe from [2] to the specific challenges in our experiment in an orthotopic pancreatic tumor xenograft mouse model for stereotactic fractionated irradiation. With this approach we aim to find the ideal field size needed for a good target coverage in addition with best sparing of normal tissue, like it is done in human radiotherapy.

Materials and methods

For estimating the needed safety margin to create a PTV in our setting of mice with orthotopic pancreatic tumors in stereotactic irradiation on a Small Animal Radiation Research Platform (SARRP, Xstrahl Ltd., Camberley, UK) we used the margin recipe introduced by van Herck et al. [2]. They separate the description of the margin for the geometrical deviations in treatment execution (which is seen as a random error) and in treatment preparation (which is seen as a systematic error). The generated formula to calculate the margin contains several factors that influence the extent of

the margin. Here, we investigated each of these factors, supported them by experiments where possible, and adjusted them to our specific preclinical setting to estimate an appropriate margin for our application.

Technical equipment

The SARRP in our institution (already described in [12,13]) is a fully integrated small animal image-guided irradiation device with cone beam computed tomography (CBCT). The gantry can be rotated by 360° and holds the dual-focus 225 kV x-ray tube (from Varian, Varian Medical Systems, Palo Alto, CA, USA). The motorized couch for specimen can be rotated and translated [14,15]. For the collimation of the beam there are a series of fixed collimators as well as a manual variable collimator. The detector on the system is a flat panel detector XRD 0822 (PerkinElmer Inc., Waltham, MA, USA) with 1024 × 1024 pixels and a native pixel size of 0.2 mm and a resolution of 0.1 mm [16].

Mice with orthotopic tumor model

All animal procedures were carried out in accordance with the local recommendations and our protocol that was officially approved according to German law for the Care and Use of Laboratory Animals and authorized by the regional government of Upper Bavaria, Germany (reference 55.2-1-54-2532-217-2015). All procedures were performed under anesthesia, and every effort was made to minimize suffering.

As previously described [9,17,18], an orthotopic xenograft pancreatic tumor mouse model in 6-week-old immunosuppressed nude mice (crl:CD1- Foxn1nu, Charles River Laboratories, Sulzfeld, Germany) was established. During a laparotomy 1.5×10^6 cells of the established human pancreatic carcinoma cell line MIA PaCa-2 (CRL-1420, ATCC, Manassas, VA, USA) were injected into the pancreatic parenchyma. The mice were immobilized with inhaled isoflurane anesthesia at a concentration of 1.5% with 4% volume of oxygen as a carrier gas during the whole procedure of imaging, treatment planning, and irradiation. The flow was adjusted to the individual need of the mice.

Experimental settings

Some of the factors in the formula for the creation of the PTV margin are dependent on the specimen, the tumor and its location as well as on the intended treatment execution settings (beam size, beam configuration, technical equipment). The basic consideration of the factors may be similar for different experiments, but each factor has to be checked for its validity by individual analyses. Our margin calculation aims to find the margin for the following situation: The above mentioned immunosuppressed nude mice with orthotopic xenograft pancreatic tumors shall be treated using the SARRP. The concept includes 5 fractions of an arc irradiation with daily contrast-agent enhanced CBCTs for image-guided RT. The planning of the treatment will be done on the first CBCT of the first fraction, which then serves as a reference.

The GTV was determined visually and contoured slice-by-slice by an experienced physician on contrast agent-enhanced CBCTs using the pre-clinical treatment planning software MuriPlan (Xstrahl Ltd., Camberley, UK, version 2.2.0). The GTV was defined as the macroscopically visible tumor tissue. By lacking further knowledge, we used the GTV also as the CTV. This is reasonable, since the potential CTV margin is small compared to the uncertainty in contouring and also small compared to the PTV margin and especially as we do not focus on the GTV/ CTV delineation, but on the creation of the PTV margin that is not dependent on the differences of GTV/ CTV.

Formula for the margin recipe

The derivation of the recipe is explained in detail in [2]. We will focus on the transition of the developed formula for the PTV margin (m_{PTV} in mm) from that work which is given (as formula number 11 in [2]) as

$$m_{PTV} = \alpha \sum + \beta\sigma - \beta\sigma_p \quad (1)$$

In formula (1), $\sum$ is the combined standard deviation (SD) of all treatment preparation (systematic) errors in mm which we will explain later on. The symbol σ stands for the SD in mm of all treatment execution (random) variations (which will be split into several factors later on), and σ_p is the width (in mm) of the penumbra.

The numerical value for factor α is dependent on the used confidence level (which percentage of patients should receive the prescribed dose) and is given in table 2 of [2]. For factor β, the numerical value is dependent on the desired dose level (the minimal dose) in the CTV and can be taken from table 3 of [2]. Both factors are numerical values without dimensions derived from theoretical considerations.

The combined SD of the treatment preparation (systematic) error $\sum$ includes several SD of preparation errors, namely $\sum_m$, $\sum_s$, and $\sum_d$, where m stands for organ motion, s for setup deviation, and d for delineation. In the case of (stereotactic) fractionation schemes, an additional term $\sum_f$ can be added to the standard expression to account for a small number of fractions n. $\sum_f$ is increasing with an increasing (random) execution error and decreasing with an increasing number of fractions. The value of $\sum_f$ can be calculated by the SD of the execution (random) error σ divided by $\sqrt{n}$:

$$\sum_f = \frac{\sigma}{\sqrt{n}} \quad (2)$$

The resulting SD is the square root of the quadratic sum of the single SDs:

$$\sum^2 = \sum_m^2 + \sum_s^2 + \sum_d^2 + \sum_f^2 \quad (3)$$

Similarly, the combination of SDs of the treatment execution (random) variations σ with σ_m (m for organ motion), σ_s (s for setup error), and σ_p (p for penumbra) is:

$$\sigma^2 = \sigma_m^2 + \sigma_s^2 + \sigma_p^2 \quad (4)$$

If (2) and (3) are inserted in (1), the formula can be written as

$$m_{PTV} = \alpha \sum + \beta\sigma - \beta\sigma_p = \alpha\sqrt{\sum_m^2 + \sum_s^2 + \sum_d^2 + \sum_f^2} + \beta\sqrt{\sigma_m^2 + \sigma_s^2 + \sigma_p^2} - \beta\sigma_p \quad (5)$$

where all factors that need to be transferred are visible.

Methods of adapting factors for the systematic error

For factor α there is no need for adjustments. As the values for that factor arise from theoretical geometrical considerations without influence of the target, the same values as stated in table 2 in [2] can be used also for our reference setup in preclinical research.

$\sum_m$ is the standard deviation for the organ motion during the treatment preparation process, in particular during the scanning process of the CBCT. This standard deviation of the organ motion itself is extremely sensitive to the particular organ (e.g. lung, pancreas, brainstem, . . .), the considered body (human, animal, individual case), and the setup in the treatment preparation situation (fixation, anesthesia, . . .). We used no fixation in terms of intrafraction motion control, but all mice were immobilized with inhaled isoflurane anesthesia. No breathing control (pressure, volume, frequency) was used. To determine the motion value, we examined the motion of the region of interest (pancreas) in three mice with fluoroscopy and quantified it using a ruler.

$\sum_s$ describes the SD from the setup error during the treatment preparation workflow on the scanner, which is determined through the precision of the scanner with the resolution of the detector. There is no additional error due to the setup of the mouse, because we use this scan as a reference for our image-guided workflow.

$\sum_d$ means the SD of the delineation error. To investigate this factor, it is important to use the appropriate image quality and the same level of experience of the user as in the real experiment. Therefore, an experienced user was delineating the tumor on one CBCT scan in our standard quality for six different mice (with a variety of tumor volumes) for three times independently, see Fig. 1 for an example. For workflow reasons the delineation was done in the Eclipse treatment planning software from Varian (Varian Medical Systems, Palo Alto, CA, USA). Afterwards, we analyzed the differences of the contours by using the Hausdorff distance. This metric investigates the distance d from each point of volume A to its nearest point in volume B. The largest value of all distances d (from all points of volume A) is the Hausdorff distance. We exported the structures as DICOM files from Eclipse and used the open source software 3D slicer version 4.11.20210226 (www.slicer.org) for evaluation. There, we used the predefined metric called 'Hausdorff distance metrics' and recorded especially the 95% Hausdorff distance. For use in our formula we averaged these values (of all three contours) per mouse and took the mean over all mice as $\sum_d$.

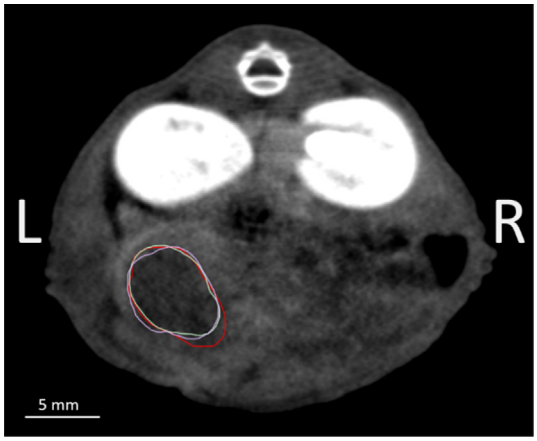

Figure 1. GTV delineated on an axial reconstructed CBCT of a mouse (kidneys with contrast agent). The same target was independently contoured several times in different colours.

$\sum_f$ accounts for the fractionation and is only of interest if the number of fractions is small. There is no dependence on the body being treated, so this formula can be used for small specimens exactly as it is used for humans and can be calculated according formula (2).

Methods of adapting factors for the random error

The adaptation of the variables for the treatment execution (random) variation σ will be described in the following. For factor β, there is no need for adjustments as the value is independent from the treated individual (human, mouse, . . .) as it only is dependent on the desired dose level in the CTV and can therefore be taken from table 3 of [2].

σ_m is the standard deviation for the organ motion during the treatment execution process. As the organ motion is the same during the execution process as during the planning process, the same value as for $\sum_m$ will be used.

σ_s describes the setup error during the treatment execution process. As our workflow involves daily image guidance, the initial setup error will be corrected by shifting the mouse after the image registration to the desired position. Any needed rotations can be performed manually on the

mouse and once more controlled with image guidance. Therefore, the setup error is theoretically negligible, except the error that is possibly introduced by the fusion process. To investigate this uncertainty, we repeated the fusion of the daily CBCT with the reference CBCT five times for five mice and recorded the required shifts. The mean shift of the 25 fusions is combined by quadratic summation with the precision of the scanner (see also description for $\sum_s$) to result in a σ_s. This does not include any change in volume or in the shape of the tumor.

σ_p, the standard deviation for the penumbra, is created from a measurement on our system with the treatment beam (arc, 10 × 10 mm^2). We irradiated a radiochromic film (Gafchromic EBT3, Ashland, Wilmington, DE, USA) in a depth similar to the interesting depth in mice and scanned it on an Epson Perfection V700 Photo scanner (EPSON Deutschland GmbH, Meerbusch, Germany) in color at 1200 dpi in transmission mode. The orientation of the film during the exposure was such that the arc rotation axis was perpendicular to the surface normal of the film. This results in the blackening of the film with a steep dose gradient in two directions of the field and less sharp dose gradients in the other directions. According to [2], $\beta \cdot \sigma_p$ is the distance between the 95% and the 50% isodose of the planned (and not blurred) dose distribution. In Fig. 2, a schematic drawing of two irradiation fields is shown together with a CTV to illustrate the influence of a sharper dose gradient (dashed line) on the coverage of the CTV (especially on the edges) following a shift.

Results

To estimate an appropriate margin for our experiment we created values for all the factors on the right side of the equation (5). In the following, we present our results for all factors, including short explanations or ranges, that may also be used in our setting. Our goal was to irradiate the CTV of at least 90% of our mice with at least 95% of the prescribed dose.

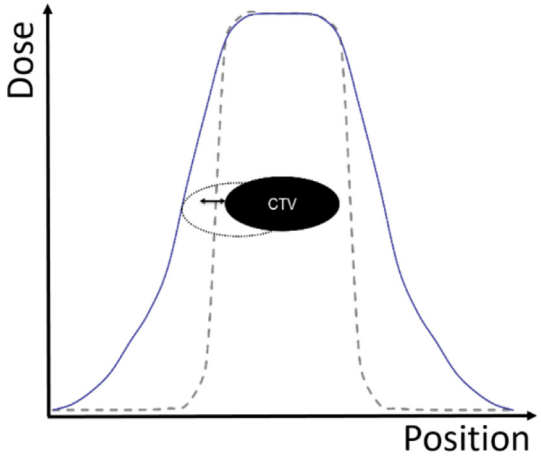

Figure 2. Two dose distributions. Dashed line indicates a sharp dose gradient, the solid line a less sharp gradient / a larger penumbra. If the shown CTV moves to the indicated place, its edge will receive a different amount of dose in both cases.

For the determination of the value for factor α (stated in table 2 in [2]), it is important to consider the dose distribution. For the stated values in this table, perfect dose conformation in 1D, 2D, or 3D is expected. As we use an arc technique (surrounding the body on the longitudinal/cranio-caudal axis), perfect conformation or the steepest dose gradient is present in the cranio-caudal direction and more or less in the other two directions. This leads, according to [2], to an approach in 3D, which yields for a confidence level of 90% the value of 2.5 for the factor α.

For the motion of the region of interest (pancreas) we found the mobility to be 0.2 to 0.4 mm, whereby the breathing cycle was around 3 s and the duration of each motion was 0.5 s. This means, the most of the time the complete area was in the desired region and only for a short proportion of time, the deviation was present. We therefore decided to take 0.3 mm for $\sum_m$ and σ_m.

The setup error during the treatment preparation $\sum_s$ is defined by the resolution of the detector of the scanner, which is the half of the pixel size, 0.1 mm.

$\sum_d$, the SD of the delineation error is set to the mean averaged Hausdorff distance (95 %) of our experiment and is found to be 0.26 mm.

Our experiment was set up with 5 fractions. $\sum_f$ can be calculated according to formula (2) by using n = 5 and the treatment execution variation σ, calculated as stated in formula (4).

Factor β is taken from table 3 (second column) of [2] for a 95% dose level and is set to 1.64.

σ_m, the standard deviation for the motion during the treatment execution process is the same value as for $\sum_m$ (0.3 mm, see above).

The setup error during the treatment execution process is defined by the resolution of the scanner (0.1 mm) and the result of our experiments for fusion uncertainty (0.26 mm) and results in 0.28 mm.

In our film measurement of the treatment beam we found two explicit different dose gradients in the different directions. In the direction where the beam enters during the arc irradiation, the dose gradient is less sharp than in the other direction (cranio-caudal, perpendicular to the plane the arc is given). As the highest risk for underdosage is certainly in the region of a high dose gradient, we used only the measurement of the sharper dose gradient for the determination of the margin. According to [2], σ_p can be determined by the distance between the 95%-Isodose and the 50%-Isodose, which is 0.8 mm. As $\beta \cdot \sigma_p = 0.8$ mm, we found $\sigma_p = 0.5$ mm.

By putting all our results of the single factors into formula (5) we gain a CTV to PTV margin of 1.5 mm for our setup. This margin is calculated for our specific experiment with mice and aims to ensure that at least 90% of the mice will receive at least 95% of the prescribed dose within the CTV. In Fig. 3 the GTV (equal to the CTV) is shown in red. The PTV with the margin of 1.5 mm is shown in green, an alternative margin of 2 mm is shown in blue.

Discussion

In the present study, we used a well-known recipe from human radiotherapy to derive a PTV margin and transferred it to our experimental setup in pre-clinical small animal irradiation of mice. Parts of the factors are strongly dependent on the exact setting of the experiment. We calculated the margin for a fractionated arc irradiation with IGRT of anaesthetized nude mice with orthotopic pancreatic tumors on our SARRP machine.

In the following we will discuss the factors of the formula needed for calculating the margin. For factor α (for the systematic errors) we took the value for 3D, even if the dose conformation is not equal in all directions. Van Herk et al. [2] proposes to consider the errors in 2D instead as 3D if for example two opposing beams are present and a dose gradient is missing in one direction. In our case with the arc irradiation there is an acceptable dose gradient in all directions, although it is not as sharp in all directions as in the cranio-caudal direction and as it is for a single beam. We decided to calculate the margin as a homogeneous margin in all directions and therefore took the sharpest dose gradient as the relevant one for the calculation of σ_p, even if perfect dose conformation is not realistic in our setting as we used a 10 by 10 mm^2 field and no individually collimated field sizes. This means that in most cases even a smaller than the calculated margin could eventually be enough to meet our criterion. We found the value σ_p for our calculation by irradiating and measuring a film with an arc beam.

Both factors for the motion uncertainty ($\sum_m$ and σ_m) are set to 0.3 mm, well-considered that there could be remarkable differences in mice, and a correction to 0.4 mm (0.2 mm) would lead to a margin that is about 0.3 mm bigger (0.2 mm smaller). The extent of the movement is not equal in all directions, but we took the most prominent movement direction for the calculation as we did not differentiate the margin itself in different orientations. This could, of course, be done in future work, but may not be feasible for every experiment and is not part of this study, even if the margin reduction could be considerable.

Delineation of the GTV is most dependent on the experience of the executing person. To achieve high quality in delineation, good imaging is important, too. This can be achieved by using multiple imaging techniques (like CT, MRI, dual-energy-CT etc. [16]) and by using contrast agents or fiducial markers [17]. In addition, a carefully selected imaging protocol, ongoing quality checks [13], and maintenance of the system can help to achieve small delineation errors. The software, especially the available tools used for contouring, are important as well for a precise and reliable contouring. A work conducted by Lappas et al. [19] found a Hausdorff distance (95%) between 0.2 mm and 0.8 mm for thoracic organs. These results for a different anatomic area are in the same magnitude as ours for the pancreas region (0.26 mm). For future, deep learning approaches, e.g. like described by Schoppe et al [20], may help to facilitate and speed up a lot of contouring work.

Our experiment schedule contains 5 fractions. If the experiment would be done with more than 5 fractions, the margin would nearly not change at all (up to about 0.2 mm reduction). If, however, the fractionation would be changed to less fractions, for example to 2 fractions, the derived needed margin would theoretically increase by about

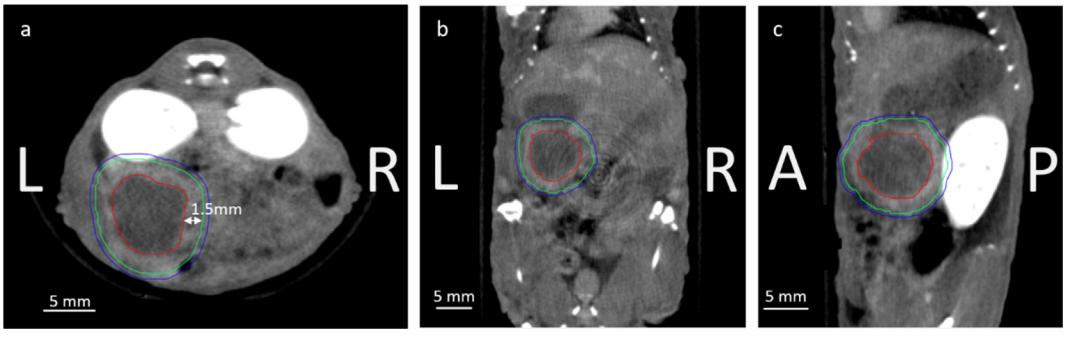

Figure 3. GTV (red) and PTV (green) on an axial (a), coronal (b), and sagittal (c) CBCT slice of a mouse. The size of the PTV margin is 1.5 mm. The blue structure shows a margin of 2 mm around the GTV. The big hyperdense organs are the kidneys with iodine-containing contrast agent.

0.3 mm. Due to the daily IGRT we directly decrease the risk and amount of setup uncertainty and nearly exclude the chance for bigger displacements, and therefore, we minimize the influence of the factor of the fractionation. So, this factor is only of minor interest for the overall margin.

The setup error in our experiment during the planning process $\sum_s$ is nearly zero due to our workflow with setting the reference to the first scan and is determined by the resolution of the scan and set to 0.1 mm. In [18], three different positioning methods are compared. The method with skin markers showed best results and minimized rotations and could be the preferred setup method. For our experiment, we investigated the setup error during the execution σ_s in a fusion study where five mice were registered several times. As we do daily IGRT for all treatments, all translations can be corrected and also remaining rotations of the mice could be corrected as they are recognized and evaluated during this registration process. Our results lead to a setup error determined by (the quadratic sum of) the error of the fusion process itself (0.26 mm) and the scanner resolution (0.1 mm). A possible deformation of the specimen cannot be corrected by this workflow, but as long as the GTV did not deform too much, translations and rotations can be sufficient to cover the target with the beam. As a major shape or volume change of the tumor for single cases cannot be predicted, this cannot and should not be part of the standard formula. If this or a different filling of variable organs nearby happens, the image guided workflow should enable an adaptive replanning of that case. An increased or decreased thickness of tissue in the path of the beam after translations and rotations may lead to increased or decreased absorption of the beam before the tumor, which cannot be avoided completely, but the usual extent can be accepted.

Some of the found values for the formula (5) to calculate the margin are not a single value but more a kind of range of values. To get a feeling of how large is the variability of the margin calculation we set all these values within its range to aim for a minimum and for a maximum margin. We ended up with 1.2 mm and 2.5 mm as minimum and maximum margins for our setup.

By comparing our results with literature, we found some descriptions of margins for small animal irradiation. For mice, we found only a few suggested values. In [21] only a GTV to CTV margin of 2 to 3 mm and no PTV (margin) was used. Kim et al. [22] proposed a margin of 2 mm for brain irradiation in mice using a Cyberknife. The size of the margin was determined in their own study by focusing on positional uncertainty. A safety margin of 2 mm was also considered in [9] to be enough from GTV to PTV in pancreatic tumors. We found in our pre-study a value of 2 mm by estimating the single values of the aforementioned formula (5). Most of the used factors could be decreased in our present study by evaluating them very precisely. Ref. [9] also shows the advantage of a single-dose SBRT versus a whole abdomen irradiation. A fractionated irradiation scheme (like we use in this study), where margins are even more important, is not shown. Also on a SARRP machine, Xu et al. [23] showed how the usage of quantitative bioluminescence tomography-guided irradiation could improve the accuracy in head (orthotopic glioblastoma) irradiation. Their suggestion is to use a 0.5 mm margin to prevent underdosage in the GTV. This value is much smaller than our margin result, but aims at a cranial irradiation and does not account e.g. for any motion uncertainties. Without accounting for motion, we would arrive at 0.9 mm with our approach. Walb et al. [24] found a safety margin of about 1.3 mm could be enough in their study for cranial irradiation in mice with cranial positioning with a stereotactic stage. The authors propose that by using this margin, the frequency of daily image guidance could be reduced significantly. The setup is not completely comparable as we did not consider cranial irradiation and had a less bony and more flexible body region. Nevertheless, the stated value of 1.3 mm corresponds very well to our result of 1.5 mm (without stereotactic stage). Walb et al. discussed that by using a margin recipe formalism, their stated margin could potentially even be reduced. Yoda and Nakagawa state in their work [25], that the used coefficient α in formula (1) could be reduced to 2.1 if anisotropic systematic positioning errors are calculated in cartesian coordinates instead of using the isotropic model in spherical coordinates. This reduction would lead to a margin of 1.2 mm in our setup. As the margin is important to have a sufficient dose coverage, it must not be defined too small. On the other hand, the bigger a margin gets, the more normal, healthy tissue will be irradiated. In Fig. 3 the green PTV is close to the kidney, whereas a CTV to PTV margin of 2 mm would result in an overlap of PTV and kidney. In a work on a phantom Vaniqui et al. [7] calculated the dose to the target and OARs in a moving lung cancer case in a phantom and found a dose difference of up to 11 % in the tumor. They concluded that new methods need to be found for tracking in such cases. A work from van der Heyden et al. [10] in the same phantom propose a simple margin recipe which takes into account the collimator size.

In addition to using the above mentioned formula, we tried to find different ways to define a PTV margin. One possibility could be the collection or creation of survival curves of populations (some with bigger, some with smaller PTV margins), but this will be very time consuming. Another approach could be to take the standard margins as for humans or to scale the ‘human’ margin. For the scaling approach it is maybe not completely clear to what the scaling should be: mouse volume, mouse weight, mouse length, PTV volume, size of organ, or different measures? But the main interest and aim of using a margin is to account for the specific uncertainties and that is why we consider the

approach to take the margin recipe and adjust it to the specific technical conditions and needs of the irradiated specimen is the best possible approach.

Note that using a precise PTV margin in the pre-clinical setting can be an additional challenge (e.g. due to missing software tools during treatment planning that could simplify creating a precise margin for the whole GTV in a few steps). Nevertheless, we think it is crucial to use a margin, especially if results of pre-clinical experiments aim to impact human radiotherapy. In some cases, already the knowledge of the importance and the size of the PTV margin may help to select a proper field size, even without explicitly contouring the PTV. We hope this work can help to reduce underdosage and uncertainty and to create awareness for the importance of using a safety margin.

Conclusion

A PTV margin is needed to ensure sufficient dose coverage of the CTV in the presence of inaccuracies or small deviations during planning or execution of the irradiation. For our small animal irradiation setting, we calculated a margin of 1.5 mm to ensure that 90% of the irradiated CTVs in our mice with orthotopic pancreatic tumors receive at least 95% of the prescribed dose. Different setups may need different margin sizes, but can be estimated according to the used formula by adapting only few of the discussed factors.

Data Availability Statement

The code used to extract the data is distributed by the authors as open-source. The patient data can be made available on request due to privacy/ethical restrictions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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