Supplement 1:

Material/Methods

CBCT Image Export, Conversion, and Import into Velocity:
For each CBCT, the reconstructed image set and the GTV structure set was saved using the MuriPlan software in the ".nrrd" format, which is DICOM compatible. The ".nrrd" files were then converted to ".dcm" files using 3D Slicer software. After exporting CBCT images from MuriPlan, it was necessary to adjust the image data due to the fact that the intensity scale of the CBCT from the SARRP is not standardized to Houndsfield Units. This causes raw images exported in MuriPlan to be completely saturated when imported into Velocity. A MATLAB script was written to reduce the intensity scale by a factor of 10 (Supplement 2) to account for this. The transformed CBCT images were then imported into Velocity, along with their corresponding GTV structure set.

Velocity Fusion and Deformable Registration and Analysis:
In Velocity, each CBCT was linked with its corresponding GTV to preserve the original location relationship between the GTV and CBCT. Scan A and its corresponding GTV represents the first treatment fraction of an fSRS course. Scan B or C represented the second treatment fraction in which the position differed from Scan A. Scan B or C was set as the primary image set in Velocity and Scan A was sent as the secondary image set. A manual rigid registration was performed on Scan A to Scan B or C followed by a deformable registration. The corresponding GTV of the deformed Scan A was resampled based on the deformation matrix used GTV. To quantify the validity of the transformed GTV structure set, the deviation of the transformed Scan A GTV centroid was compared to the contoured GTV centroid from Scan B and C. The deviation was measured in all three planes.

Import Deformed CBCT and GTV into MuriPlan
To demonstrate proof of concept, DICOM exports were performed from Velocity of each transformed GTV from Scan A in relationship to Scan B and C. These images were imported into MuriPlan and overlaid with the original GTV contour for that animal and position.

Variation between GTVs as identified by MRI-CBCT Rigid Registration versus Velocity Adaptive Fusion.
To assess for degree of variation between the two methods, we compared scans for groups B and C (scans in to prone position versus supine, respectively). Although the mice in groups B and C were in different positions, the absolute GTV centroid shifts were recorded and compared to minimize the variations in analysis were additional registration of CBCT scans from B to C performed.

Results

Inter-Operator Variation
In order to address the variation in GTV delineation between operators, the centroid distance was measured in 3 planes between GTVs contoured based on MR fusion by two different operators to the same CBCT scan. From this a vector in 3 planes was calculated based on GTV positioning distances for 12 separate CBCT scans (3 scan positions for 4 different mice) evaluated by 2 separate operators. Average shifts in 3-planes as well as vector distances are shown in Supplemental Table 1 for mice in the prone position (scans A+B) and the supine position (scans C), respectively.

Intra-Operator Variation

To address the inherent error introduced by a single operator performing multiple MR fusions and GTV delineations in Muriplan, we calculated the GTV centroid shifts in 3 planes, as well as the vector shift by comparing two mice placed in the prone position (Scan A versus B). GTVs from Scan A were rigidly fused to Scan B. MR-fusion derived GTVs created in Scan B were compared to the rigidly fused GTVs from Scan A. Average shifts in 3-planes as well as vector distances are shown in Supplemental Table 2 for mice in the prone position comparing GTVs.

Dosimetry Analysis

An important question is to assess the quality of GTV coverage from adaptive planning as compared treatment plans developed from rigid MRI to CBCT registration and GTV identification. Two AP-PA plans were generated using 3 mm and 5 mm fixed cone targeting the velocity-based GTV to assess radiation dose coverage for all mice in both shifted scan positions (scans A to B and A to C). These plans were used to assess coverage for GTV determined by a rigid MRI to CBCT registration (Supplement Figure 1). The 3 mm cone plan offered acceptable coverage of GTV. A 5 mm cone plan was compared to represent a 1 mm symmetric expansion of the treatment area. This demonstrated that adequate coverage (based on the 90% prescription Isodose coverage) was achieved in all cases for 3 mm cones, but that superior coverage was achieved when expanding the treatment field to 5 mm cones. We recommend that this be done when superior target coverage is vital to the study and nearby organs at risk (OAR) are not a major concern. Supplement Figure 1 provides a visual representation of this method while Supplement Table 3 provides details on the dosimetric analysis results.

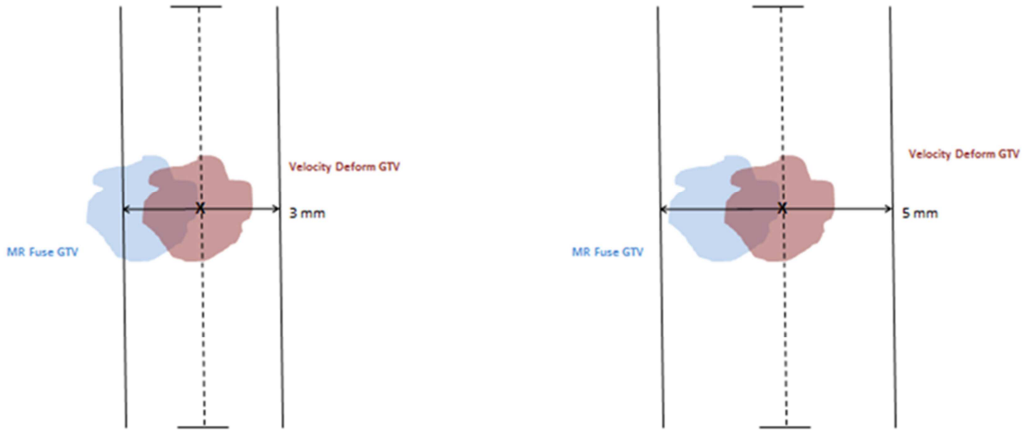
Supplement Table 1. Inter-Operator GTV Centroid Shifts in 3 Planes (mean ± SEM, mm).

Setup	Axial	Sagittal	Coronal	Total Vector
Supine	0.48±0.06	0.60±0.11	0.49±0.06	0.96±0.08
Prone	0.38±0.14	0.77±0.0.05	0.60±0.11	1.09±0.07

Supplement Table 2. Intra-Operator GTV Centroid Shifts in 3 Planes (mean ±SEM, mm).

Operator	Axial	Sagittal	Coronal	Total Vector
1	0.76±0.13	0.69±0.13	0.59±0.06	1.19±0.16
2	0.30±0.12	0.30±0.11	0.24±0.13	0.50±0.19
Combined	0.53±0.12	0.49±0.11	0.41±0.09	0.85±0.17

Supplement Figure 1. Visual representation of the treatment planning method used to evaluate dosimetric quality of target coverage.



Supplement Table 3: Dosimetric Analysis of MRI-Fused GTV Coverage Based On Adaptive Registered GTV Targeting

	Mean Dose (% Prescription Dose)	Minimum Dose (% Prescription Dose)	%Volume of 90% prescription dose
3 mm	101.94±1.46	83.27±8.50	98.51±0.66
5 mm	102.80±1.56	95.07±0.98	100.00±0.00