Supplementary materials and methods

Alanine dosimetry

We applied cylindrical alanine pellets (diameter 4.8 mm and height 2.7 mm) from Harwell Dosimeters Ltd, UK. Seven pellets were stacked on top of each other and placed in an Eppendorf tube. The tubes with pellets were positioned inside the foam holder in the area of mouse oral cavity and irradiated with the same settings and dose per fractions as mice in all treatment groups (X-rays, BP, TM). The pellets were read out using a Bruker EMX micro spectrometer, where the specific read out procedure is described in [1]. The system was calibrated in a cobalt-60 field with traceability to PTB in Germany. Beam quality correction factor (k_Q) relative to cobalt for the 100 kV x-ray beam was estimated to be $k_Q = 1.43$ with an uncertainty $u(k_Q)=0.14$ (k=1) based on [2,3]. Beam quality correction factors for the proton irradiations were estimated to be $k_Q = 1.022$ for the plateau and $k_Q = 1.033$ for the measurements in or near the Bragg peak based on [4,5]. The uncertainty of the proton beam-quality corrections was estimated to $u(k_Q)=0.02$ (k=1). Depth dose distributions for all experiments were plotted using D_W and position of the pellet in the stack, where the pellet thickness was translated into water equivalent thickness (WET) [6].

Monte Carlo simulations

Monte Carlo (MC) simulations of the three-dimensional (3D) dose distribution in mice were conducted in FLUKA 4–3.2 [7,8] and Flair 3.2-4.3 [9]. Briefly, the simulations were performed in computed tomography (CT) images of one euthanized male mouse (11 weeks old). For the X-ray irradiation setup, a 100 kV spectrum attenuated through a 2 mm aluminum filter was simulated. For the proton irradiation setup, nominal energy of 24 MeV and 60 MeV with a Gaussian energy distribution of 3 MeV and 2 MeV full width at half maximum (FWHM) spread were simulated, respectively. The MC simulations were run using standard FLUKA physics settings (cf. PRECISIOn defaults in the FLUKA manual). The absorbed dose was scored on a voxel-by-voxel basis (using the USRBIN option in FLUKA). The mean LET in the irradiated part of the mouse was crudely estimated to be 3.3 and 4.2 keV/µm for TM and BP, respectively, while it was around 1.0 keV/ µm for X-rays. It is stressed the local LET approximately mid-plane in the animal will be much higher for the BP approach.

References

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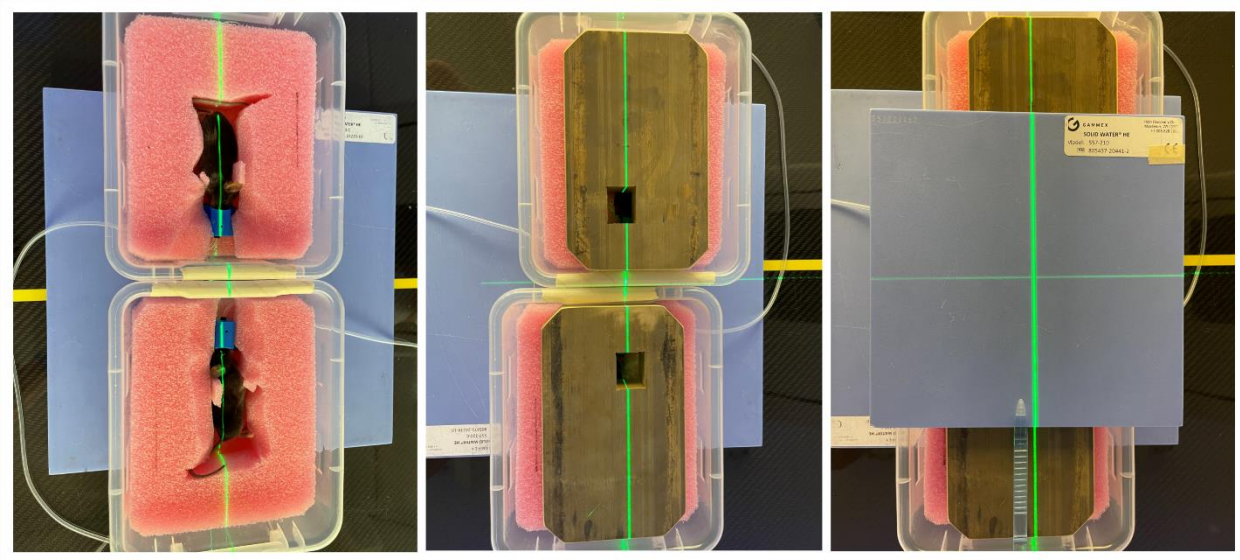
Supplementary figures and tables



Supplementary Figure 1. Pictures of the irradiation set-up for proton irradiation.

Supplementary Table 1: Dermatitis scoring table based on CTC v. 2.0

Dermatitis score	Observed effects
0 None	No change over baseline
1 Mild	Faint erythema, mild edema and dry desquamation
2 Moderate	Bright erythema, moderate edema and patchy moist desquamation
3 Severe	Severe erythema and edema, confluent moist desquamation
4 Life threatening	Ulceration, necrosis, hemorrhage

Supplementary Table 2: Mucositis scoring table based on CTC v. 2.0

Mucositis score	Observed effects
0 None	No change over baseline
1 Mild	Erythema
2 Moderate	Patchy mucositis
3 Severe	Confluent, fibrinous mucositis
4 Life threatening	Ulceration, necrosis, hemorrhage

Supplementary Table 3: Individual raw data

Mouse ID		Saliva volume (microliter)			Mucositis on the tongue											
Original	Manuscript	Baseline	Day 9/12	Day 35	Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 28	Day 31	Day 35
11-1	X-rays 1	35	25	20	0	0	0	1	0	0	0	0	0	0	0	0
11-3	X-rays 2	60	30	10	0	0	0	2	2	0	0	0	0	0	0	0
11-6	X-rays 3	80	40	15	0	0	0	2	2	2	2	2	1	0	0	0
11-7	X-rays 4	105	2	-	0	0	2	2	2	2	-	-	-	-	-	-
11-9	X-rays 5	70	20	20	0	0	0	2	1	1	2	0	0	0	1	0
12-1	X-rays 6	35	40	45	0	0	0	2	2	0	0	0	0	0	0	0
12-4	X-rays 7	200	60	45	0	0	0	0	2	2	2	2	2	2	2	2
12-5	X-rays 8	155	65	60	0	0	0	0	2	2	2	2	1	2	2	0
12-8	X-rays 9	150	50	100	0	0	0	0	0	0	0	0	0	0	0	0
12-10	X-rays 10	100	30	50	0	0	2	2	2	2	2	2	2	0	0	0
15-11	TM1		62	30	2	2	3	3	3	1	0	0	0	0	0	0
15-12	TM2		60	40	2	2	3	3	2	0	0	0	0	0	0	0
16-6	TM3	140	13	10	2	2	3	3	2	1	0	0	0	0	0	0
16-7	TM4	155	62	10	1	2	3	3	2	0	0	0	0	0	0	0
18-1	TM5		70	-	0	2	3	3	1	0	-	-	-	-	-	-
18-2	TM6		7	-	0	2	-	-	-	-	-	-	-	-	-	-
18-3	TM7		56	7	2	2	3	3	1	1	0	0	0	0	0	0
18-4	TM8		16	60	1	2	3	3	1	0	0	0	0	0	0	0
15-2	BP1	100	28	-	1	2	3	3	2	2	-	-	-	-	-	-
15-3	BP2	130	75	-	1	1	3	3	2	1	-	-	-	-	-	-
15-4	BP3	70	25	150	1	2	3	3	2	1	0	0	0	0	0	0
15-5	BP4	35	92	-	1	2	3	-	-	-	-	-	-	-	-	-
15-6	BP5	60	80	110	1	2	3	3	1	1	0	0	0	0	0	0
15-7	BP6	20	25	-	1	1	3	-	-	-	-	-	-	-	-	-
15-8	BP7	90	22	50	1	1	3	3	2	1	0	0	0	0	0	0
15-9	BP8	72	105	85	1	2	3	3	2	1	0	0	0	0	0	0
16-1	BP9	160	17	-	1	1	3	-	-	-	-	-	-	-	-	-
16-2	BP10	133	65	45	1	1	3	3	1	1	0	0	0	0	0	0

15-1	Control protons 1	70	82	-	0	0	0	0	0	0	-	-	-	-	-	-	
16-3	Control protons 2	175	97	35	0	0	0	0	0	0	0	0	0	0	0	0	
16-4	Control protons 3	100	112	160	0	0	0	0	0	0	0	0	0	0	0	0	
16-5	Control protons 4	30	172	155	0	0	0	0	0	0	0	0	0	0	0	0	
16-8	Control protons 5	172	77	190	0	0	0	0	0	0	0	0	0	0	0	0	
17-1	Control protons 6	110	152	190	0	0	0	0	0	0	0	0	0	0	0	0	
17-10	Control protons 7	135	240	150	0	0	0	0	0	0	0	0	0	0	0	0	
11-2	Control X-rays 1	120	160	60	0	0	0	0	0	0	0	0	0	0	0	0	
11-4	Control X-rays 2	65	90	70	0	0	0	0	0	0	0	0	0	0	0	0	
11-5	Control X-rays 3	20	60	55	0	0	0	0	0	0	0	0	0	0	0	0	
11-8	Control X-rays 4	105	45	60	0	0	0	0	0	0	0	0	0	0	0	0	
11-10	Control X-rays 5	90	90	105	0	0	0	0	0	0	0	0	0	0	0	0	
12-2	Control X-rays 6	110	140	-	0	0	0	0	0	0	-	-	-	-	-	-	
12-3	Control X-rays 7	115	100	110	0	0	0	0	0	0	0	0	0	0	0	0	
12-6	Control X-rays 8	160	125	130	0	0	0	0	0	0	0	0	0	0	0	0	
12-7	Control X-rays 9	170	60	120	0	0	0	0	0	0	0	0	0	0	0	0	
12-9	Control X-rays 10	95	120	105	0	0	0	0	0	0	0	0	0	0	0	0	

Abbreviations: TM = Transmission Mode proton treatment plan. BP = Bragg Peak proton treatment plan

Dermatitis on the lip

Day 8	Day 10	Day 12	Day 14	Day 16	Day 18	Day 20	Day 22	Day 24	Day 28	Day 31	Day 35
1	1	1	2	2	3	3	2	1	1	0	0
1	1	1	1	1	2	2	2	2	1	0	0
1	1	1	1	2	3	3	2	2	1	0	0
0	1	1	2	2	3	-	-	-	-	-	-
1	1	1	2	2	3	3	2	1	1	0	0
1	1	1	1	2	3	3	2	2	1	0	0
1	1	1	1	2	3	3	2	1	1	0	0
0	1	1	1	2	2	2	2	1	1	0	0
1	1	1	1	1	1	1	1	1	1	0	0
1	1	1	2	2	2	2	2	2	1	0	0
1	1	2	3	3	3	2	1	1	0	0	0
1	1	2	3	3	3	2	1	1	0	0	0
1	1	1	2	3	3	2	1	1	0	0	0
1	1	1	2	2	2	1	1	1	0	0	0
1	1	1	2	3	3	-	-	-	-	-	-
1	1	-	-	-	-	-	-	-	-	-	-
1	1	1	2	2	2	2	1	1	0	0	0
1	1	2	2	3	2	1	1	1	0	0	0
1	1	2	2	3	3	-	-	-	-	-	-
1	1	2	2	3	3	-	-	-	-	-	-
1	1	2	2	3	3	2	1	1	0	0	0
1	1	2	-	-	-	-	-	-	-	-	-
1	1	1	2	2	2	1	1	1	0	0	0
1	1	1	-	-	-	-	-	-	-	-	-
1	1	1	2	2	3	2	1	1	0	0	0
1	1	1	2	2	2	1	1	1	0	0	0
1	1	1	-	-	-	-	-	-	-	-	-
1	1	1	1	2	2	2	1	1	0	0	0

0	0	0	0	0	0	-	-	-	-	-	-
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
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0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	-	-	-	-	-
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0