Supporting Information

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SI Materials and Methods

S1. The treated pouch was periodically everted under light i.p. ketamine (70 mg/kg bw)/xylazine (10.5 mg/kg bw) anesthesia and examined to monitor tumor development. When the exophytic tumors, i.e., squamous cell carcinomas, had developed and reached a diameter of ∼3–5 mm, the animals were used for in vivo BNCT studies. A variable number of tumors develop in each cancerized pouch.

S2. MAC-TAC liposome suspensions were administered i.v. at a dose of 18 mg ^{10}B per milligram bw (∼1.39 mL/100 g bw) as i.v. bolus injections in the surgically exposed jugular vein of animals (120–170 g bw) anesthetized with an i.p. injection of ketamine (70 mg/kg bw)/xylazine (10.5 mg/kg bw), followed by skin suture. Because the liposome suspension is stored at 5 °C, care was taken to administer the suspension only after it had reached room temperature. The suspension was mixed gently before loading the syringe.

S3. A tunnel penetrating the graphite structure of the thermal column enables the insertion of samples into a near-isotropic neutron field while the reactor is in normal operation. The neutron field is very well thermalized, the ratio between epicadmium to thermal flux being less than 0.05%. This characteristic makes the radiation dose component from hydrogen recoil (i.e., fast neutron dose) in tissue negligible. Total dose can then be considered as the sum of boron dose (when ^{10}B is administered), ^{14}N capture dose, and γ-dose. A shield was constructed to protect the body of the animal from the thermal neutron flux while exposing the everted cheek pouch bearing tumors. The enclosure held two hamsters and was fabricated from plates composed of a 6-mm layer of lithium carbonate enriched to 95% in ^{6}Li, sealed within sheets of Lucite. The hamster pouch was everted out of the enclosure onto a protruding shelf. The thermal neutron flux at all locations within the shield container was at least a factor of 20 lower than the flux on the pouch shelf. The thermal neutron flux is approximately 9.1×10^{9} n·cm^{-2}·s^{-1} in the outermost position on the pouch shelf and 7.7×10^{9} n·cm^{-2}·s^{-1} in the center position. These values are ∼25% lower than the free flux at this location, largely as a result of local flux depression by the shield enclosure.

S4. The severity of mucositis was evaluated semiquantitatively in dose-limiting precancerous tissue as previously described (1) according to an oral mucositis scale based on macroscopic features, adapted for carcinogen-treated hamster cheek pouch from the WHO classification for oral mucositis in human subjects (2) and the six-point grading system for normal hamster cheek pouches of Sonis et al. (3). Grade 0 represents healthy appearance with no erosion or vasodilation; grade 1 represents erythema and/or edema and/or vasodilation with no evidence of mucosal erosion; grade 2 represents severe erythema and/or edema, vasodilation, and/or superficial erosion; grade 3 represents severe erythema and/or edema, vasodilation, and formation of ulcers <2 mm in diameter; grade 4 represents severe erythema and/or edema, vasodilation, and formation of ulcers ≥2 mm and <4 mm in diameter; and grade 5 represents severe erythema and/or edema, vasodilation, and formation of ulcers ≥ 4 mm in diameter or multiple ulcers ≥ 2 mm in diameter and/or necrosis area ≥ 4 mm in diameter. Grading was based on the most severe feature observed, avoiding areas in close vicinity of persistent tumors and the pouch cul-de-sac that is histologically different from the rest of the pouch, overly radiosensitive, and of limited clinical relevance.

S5. Very importantly, here we must stress that the carcinogenesis protocol used herein for tumor control studies is too aggressive to allow for an adequate long-term follow-up of the inhibitory effect of BNCT on tumor development from precancerous tissue. The precancerous tissue induced by this aggressive carcinogenesis protocol does not adequately mimic the less aggressive field cancerized human oral mucosa. That would require the use of a less aggressive carcinogenesis model that we previously developed to be used specifically for the long-term study of precancerous tissue (4). Because that model cannot be used for tumor control studies and was not used in the present study, the data we present herein on the inhibitory effect of the different BNCT protocols on the development of novel tumors from precancerous tissue must be interpreted only as an additional comparative end point to evaluate therapeutic effect; the actual values must not be considered clinically relevant because they refer to an overly aggressive precancerous tissue (1).

SI Results

S6. The preliminary study at 3.5 Gy showed only the mildest mucositis in precancerous tissue (grade 1) and an OR rate of 69%. The preliminary studies at 6 Gy and 7 Gy showed severe mucositis in precancerous tissue (grade 4 and grade 5 with tissue loss, respectively) that peaked at 2 wk post-BNCT. In the remaining pouch tissue available for evaluation, OR rates were 89% and 73%, respectively.

As mentioned in *S3*, only ^{10}B (when administered), ^{14}N capture, and γ-dose contribute to total dose. Irradiation times were calculated to deliver the prescribed doses assuming the specified boron content in precancerous tissue. Doses to different tissues were then assessed taking into account the corresponding boron contents and a zero boron content for beam-only groups.

S7. The relative scarcity of the overly large tumors (>100 mm^3) in the hamster cheek pouch model of oral cancer complicates the analysis of response in this particular group of tumors. We can only mention that some cases of CR in large tumors were observed only with the double-application protocols, whereas the single-application protocols failed to induce CR in large tumors. In the case of the more numerous group V (single-application BNCT at 5 Gy), in which a statistical analysis of the group of nine large tumors was possible, CR rate was significantly higher in small tumors than in large tumors ($P = 0.0082$). In the case of the beam-only group, CR was seen only in small tumors.

1. Monti Hughes AM, et al. (2013) Boron neutron capture therapy for oral precancer: Proof of principle in an experimental animal model. *Oral Dis* 19(8):789–795.
2. López-Castaño F, Oñate-Sánchez RE, Roldán-Chicano R, Cabrerizo-Merino MC (2005) Measurement of secondary mucositis to oncohematologic treatment by means of different scale. Review. *Med Oral Patol Oral Cir Bucal* 10(5):412–421.
3. Sonis ST, et al. (2000) Defining mechanisms of action of interleukin-11 on the progression of radiation-induced oral mucositis in hamsters. *Oral Oncol* 36(4):373–381.
4. Heber EM, et al. (2010) Development of a model of tissue with potentially malignant disorders (PMD) in the hamster cheek pouch to explore the long-term potential therapeutic and/or toxic effects of different therapeutic modalities. *Arch Oral Biol* 55(1):46–51.

Table S1. Percentage of tumor response 4 wk after the end of the treatment protocol

Group/outcome	3.5 Gy	6 Gy	7 Gy	4 Gy	5 Gy	5 Gy/4 wk/5 Gy	5 Gy/6 wk/5 Gy	5 Gy/8 wk/5 Gy	Beam only	Control
Hamsters	2	2	2	5	25	5	4	6	6	12
Tumors	17	17	11	57	102	27	19	16	36	77
Maximum mucositis, grade	1	4	5	2	2	2	2	1	1	1
PR, %	62	24	45	28	37	19	42	38	14	16*
$PR_{0.5}$, %	38	18	27	14	30	15	37	25	3	0
CR, %	8	65	28	42	33	52	37	50	14	0
NR, %	31	11	28	23	29	30	21	13	72	84
OR, %	69	89	73	70	71	70	79	88	28	16*

Neutron irradiation dose to precancerous tissue quoted in Grays. Interval between BNCT treatments in weeks in the case of repeat treatment. NR, no tumor response (tumors continued to grow); $PR_{0.5}$, reduction to ≤50% of initial tumor volume.
*Spontaneous reduction in size.

Table S2. Tumor response 4 wk posttreatment for various tumor sizes

Tumors	No. of tumors	CR, %	PR, %	NR, %	OR (PR + CR), %
Single application 5 Gy BNCT, 4 wk post BNCT					
Total	102	33	37	29	71
Large: >100 mm^3	9	0	63	38	63
Medium: 10–100 mm^3	37	19	60	21	79
Small: <10 mm^3	56	48	18	34	66
5 Gy–8 wk–5 Gy, 4 wk post second application BNCT					
Total	16	50	38	13	88
Large: >100 mm^3	3	33	67	0	100
Medium: 10–100 mm^3	7	29	57	14	86
Small: <10 mm^3	6	67	17	17	83
5 Gy–6 wk–5 Gy, 4 wk post second application BNCT					
Total	19	37	42	21	79
Large: >100 mm^3	0				
Medium: 10–100 mm^3	8	13	75	13	88
Small: <10 mm^3	11	55	27	18	82
5 Gy–4 wk–5 Gy, 4 wk post second application BNCT					
Total	27	52	19	30	70
Large: >100 mm^3	1	0	100	0	100
Medium: 10–100 mm^3	8	38	38	25	75
Small: <10 mm^3	18	61	6	33	67
4 Gy–4 wk–4 Gy, 4 wk post second application BNCT					
Total	41	41	22	37	63
Large: >100 mm^3	1	0	100	0	100
Medium: 10–100 mm^3	10	50	30	20	80
Small: <10 mm^3	30	47	17	37	63
Beam only, 4 wk post irradiation					
Total	36	14	14	72	28
Large: >100 mm^3	1	0	0	100	0
Medium: 10–100 mm^3	11	0	27	73	27
Small: <10 mm^3	24	21	8	71	29

Neutron irradiation dose to precancerous tissue shown in Grays. NR, no tumor response (tumors continued to grow).