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Original Article

Radiation enhancement using focussed ultrasound-stimulated microbubbles for head and neck cancer: A phase 1 clinical trial

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ABSTRACT

Background and purpose: Preclinical research demonstrated that the exposure of microbubbles (intravascular gas microspheres) to focussed ultrasound within the targeted tumour upregulates pro-apoptotic pathways and enhances radiation-induced tumour cell death. This study aimed to assess the safety and efficacy of magnetic resonance (MR)-guided focussed ultrasound-stimulated microbubbles (MRgFUS-MB) for head and neck cancers (HN).

Materials and methods: This prospective phase 1 clinical trial included patients with newly diagnosed or recurrent HN cancer (except nasopharynx malignancies) for whom locoregional radiotherapy with radical- or palliative-intent as deemed appropriate. Patients with contraindications for microbubble administration or contrast-enhanced MR were excluded. MR-coupled focussed ultrasound sonicated intravenously administered microbubbles within the MR-guided target volume. Patients receiving 5–10 and 33–35 radiation fractions were planned for 2 and 3 MRgFUS-MB treatments, respectively. Primary endpoint was toxicity per CTCAEv5.0. Secondary endpoint was tumour response at 3 months per RECIST 1.1 criteria.

Results: Twelve patients were enrolled between Jun/2020 and Nov/2023, but 1 withdrew consent. Eleven patients were included in safety analysis. Median follow-up was 7 months (range, 0.3–38). Most patients had oropharyngeal cancer (55 %) and received 20–30 Gy/5–10 fractions (63 %). No systemic toxicity or MRgFUS-MB-related adverse events occurred. The most severe acute adverse events were radiation-related grade 3 toxicities in 6 patients (55 %; dermatitis in 3, mucositis in 1, dysphagia in 6). No radiation necrosis or grade 4/5 toxicities were reported. 8 patients were included in the 3-month tumour response assessment: 4 had partial response (50 %), 3 had complete response (37.5 %), and 1 had progressive disease (12.5 %).

Conclusions: MRgFUS-MB treatment was safe and associated with high rates of tumour response at 3 months.

Introduction

The standard treatment for unresectable locally advanced head and neck (HN) cancer involves high-dose radiation (typically at 70 Gy in 33–35 fractions) with concurrent platinum-based chemotherapy [1–3]. Chemotherapy serves as a radiosensitizer, and meta-analyses of randomized clinical trials have shown improved overall survival compared to radiation alone [1,2]. However, this approach is associated with increased toxicity due to chemotherapy's non-selective nature [1,2]. Moreover, despite combined treatment, locoregional relapse remains the most common treatment failure in approximately one-third of patients [1,2,4]. Thus, there is an unmet need for novel methods to

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selectively enhance radiotherapy efficacy without increasing toxicity.

Microbubbles are encapsulated gas spheres (<10 μm) predominantly used as ultrasound contrast agents. However, in recent years, significant research has explored the combination of focussed ultrasound (FUS)-stimulated microbubbles for potential cancer-related applications [5–16]. When intravascular microbubbles are exposed to FUS waves, they rapidly expand and collapse, causing shock waves that disrupt endothelial cells, leading to a temporary and reversible increase in the permeability of blood vessels [5]. This process may increase the intake of therapeutic agents like chemotherapy at the target tumour or within the central nervous system by opening blood–brain barrier, or facilitate liquid biopsy [8,15,16].

Moreover, comprehensive research has also demonstrated that disruption of endothelial cells activates the acid sphingomyelinase (ASMase)-ceramide pathway, which results in endothelial cell apoptosis [6,8]. Interestingly, these same pro-apoptotic pathways are triggered by high radiation doses (>8–10 Gy per fraction) and contribute to the ablative effect of stereotactic body radiotherapy [6–12,17]. A number of preclinical studies [6–14,17] demonstrated that combining ultrasound-stimulated microbubbles with radiation therapy upregulate these pro-apoptotic pathways, resulting in a several-fold increase in radiation-induced tumour cell death. For instance, studying small animal tumour models, Czarnota et al. [12] observed 4 % (±2 %) of tumour cell death with a single 2 Gy fraction, compared to 44 % (±13 %) and 70 % (±8 %) when FUS-stimulated microbubbles were combined with a 2 Gy and 8 Gy fraction, respectively.

Subsequently, a system that integrates a FUS device into the couch of a magnetic resonance (MR) platform (Profound Medical/Philips Sonalleve, Mississauga, Canada/Philips Healthcare, Best, Netherlands) became commercially available. This system improves the visualization of the target tumour and allows FUS to be guided by MR imaging. While the FUS can operate under various conditions to induce hyperthermia, it was recalibrated in our department to operate at optimal parameters for inducing bubble cavitation without causing heat or tissue damage [7,10–14]. We then studied this clinical system in large animal tumour models and confirmed the efficacy of MR-guided FUS-stimulated microbubbles (MRgFUS-MB) in enhancing radiation-induced tumour cell kill compared with radiation alone [13,14].

Encouraged by promising results from these preclinical studies, we simultaneously conducted two independent phase I clinical trials to evaluate the safety and effectiveness of using MRgFUS-MB treatment for breast and HN cancer. In our recently completed clinical trial for breast malignancies, we observed no relevant adverse events and noted encouraging tumour responses with MRgFUS-MB treatment [18,19]. In the present study, our primary goal is to report the safety of this innovative selective radioenhancement therapy specific for HN cancer.

Methodology

Study design and participants

This single-center investigator-initiated phase 1 clinical trial aimed at assessing the safety and effectiveness of combining MRgFUS-MB, a radioenhancement therapy, with radiation regimens deemed suitable for treating HN cancer. We aimed to enroll 20 patients with primary HN malignancies who were referred for locoregional radiotherapy at Sunnybrook Health Sciences Centre, and this report constitutes a preliminary analysis conducted after the enrollment of 12 patients. Patients were eligible if they were older than 18 years; had newly diagnosed or recurrent squamous cell carcinoma of the larynx, oropharynx, hypopharynx, oral cavity, salivary glands, or paranasal sinuses; and were planned for locoregional radiotherapy with any radical- or palliative-intent regimen as deemed adequate by a multidisciplinary team or standard practice. Patients with nasopharynx cancer were not included in our study as they are typically managed with induction chemotherapy rather than upfront concurrent chemoradiation. Patients who had contraindications to contrast-enhanced MR (such as those with metallic implants) or microbubble administration (including prior allergic reactions or significant comorbidities like cardiac insufficiency), abnormal coagulation profiles or impaired liver/renal function, who weighed over 140 kg, had significant ulceration or bleeding at the target lesion, were using anticoagulants, or had an Eastern Cooperative Oncology Group (ECOG) performance status of ≥3 were excluded from the study. The research adhered to good clinical practice guidelines and followed the principles of the Helsinki declarations. All participants in the study provided written consent before participating. The study protocol received approval from the institutional research ethics committee at Sunnybrook Health Sciences Centre (protocol number 076-2019) and was registered on clinicaltrials.gov (identifier NCT04431648) in June 2020. Demographic and clinical data were collected from institutional electronic medical records.

Magnetic resonance-guided focussed ultrasound platform

A commercially available MRg-FUS platform (Profound Medical/ Philips Sonalleve, Mississauga, Canada/ Philips Healthcare, Best, Netherlands) was used for the purpose of this study. The MR system consisted of a Philips Ingenia Elition X system (Philips Healthcare, Netherlands) featuring a 70 cm bore and a magnetic field strength of 3.0 T. The FUS was configured to operate with a frequency of 0.8 MHz, a maximum power output of 7.3 W, and a peak negative pressure of 570 kPa not to cause heat nor tissue damage. These specific FUS settings were selected based on prior preclinical data indicating they are optimal for inducing microbubble cavitation and enhancing radiation efficacy [7,10–14].

Procedures

Radiotherapy and MRgFUS-MB treatments were conducted as outpatient procedures. The treating radiation oncologist decided on the radiation target volume and treatment regimen per standard of care, without interference from the research team. All patients underwent standard computed tomography simulation, and additional MR imaging simulation [20] for radiation planning was at the treating physician's discretion. Radiotherapy was administered using computed tomography image-guided linear accelerators and inverse-planning intensity-modulated radiotherapy (IMRT) or volumetric modulated arc therapy (VMAT), as per our institutional standard practice.

The number of MRgFUS-MB treatments depended on the radiotherapy regimen (Fig. 1A). Patients undergoing 5–10 radiation fractions were scheduled to undergo a total of 2 MRgFUS-MB sessions distributed before fraction 1 and 5. This strategy aligns with the methodology used in some of our preclinical studies and is justified by the observation that an interval of 5 days after the first MRgFUS-MB treatment allows for a significant reduction in tumour vascularity and volume, at which point a second radioenhancement treatment was administered [12,14,21]. For patients undergoing 33–35 radiation fractions, administering 1 MRgFUS-MB treatment every 5 radiotherapy fractions could reduce treatment adherence. Therefore, we arbitrarily assigned 3 MRgFUS-MB sessions throughout the radiation course, scheduled before fractions 1, 16, and 30.

The MRgFUS-MB treatment technique is illustrated in Fig. 1B [17,18,22]. Patients were positioned on the MR bed with the target tumour in contact with an ultrasound gel pad (Aquaflex; Parker, Hannover, Germany), which was placed on top of the FUS transducer to facilitate contact without air gaps. MR imaging of the region of interest was acquired with the patient in the treatment position and used by the radiation oncologist to define the FUS-target volume and place individual ultrasound treatment cells (cylindrical shape, 2.8 cm [height] × 1 cm [diameter]) that covered the entire treatment volume. The number of treatment cells varied depending on the tumour size. Commercially available microbubbles (DEFINITY®, Lantheus Medical) [23] were

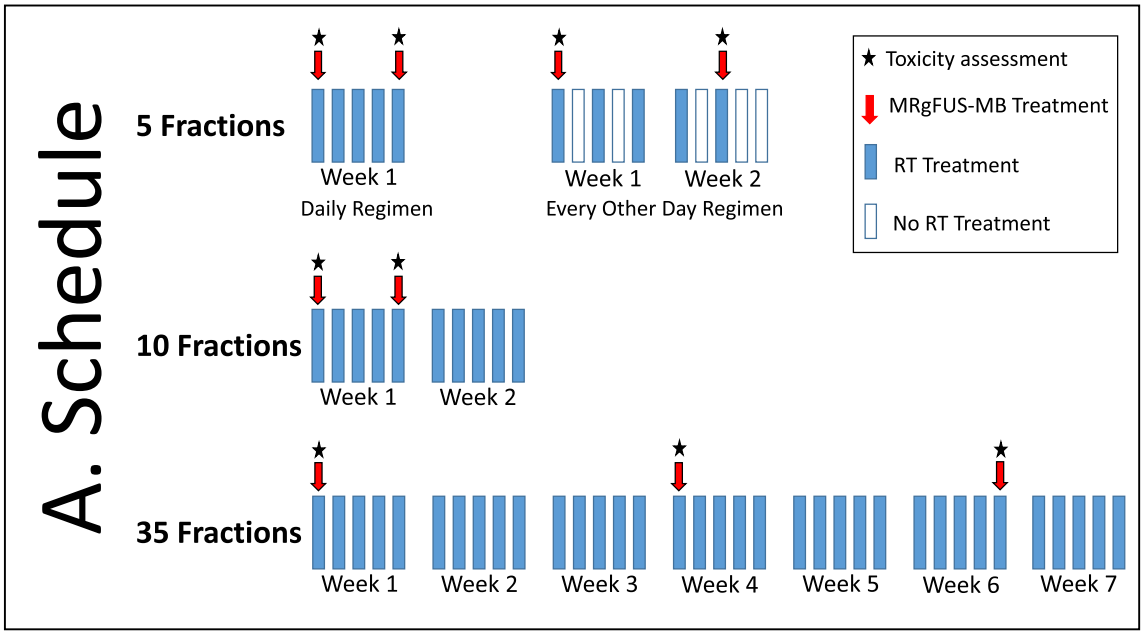

Fig. 1. A. Schedule and B. Technique of magnetic resonance-guided focussed ultrasound-stimulated microbubble treatment. B1. Patient's Setup on the MR-Guided Focussed Ultrasound Platform: The MR-guided focussed ultrasound platform consists of a focused ultrasound system incorporated into the magnetic resonance flat table. Patients are positioned so that the target tumour makes direct contact with a gel pad placed over the ultrasound transducer. The positioning is individualized for each patient, aiming to achieve the most comfortable position possible. B2. MR-guided Focussed Ultrasound Planning: The treating radiation oncologist utilizes magnetic resonance imaging to identify the target tumour (represented in pink) and place individual cylindrical ultrasound cells to cover the entire target volume (four ultrasound cells were represented in yellow). B3. Mechanical Agitation of Microbubbles: Microbubbles (encapsulated gas microspheres <10 μm) undergo mechanical agitation using a Vialmix unit for 45 s. Then, 1 mL solution of the product is administered intravenously followed by a saline flush before each ultrasound treatment cell. B4. Biophysical Effects of Stimulated Microbubbles in the Vasculature of the Target Tumour: The ultrasound cells are activated sequentially until the entire target tumour has been treated. One particular ultrasound cell is illustrated in green. When intravascular microbubbles are exposed to focussed ultrasound waves, they rapidly expand and collapse, causing shock waves that disrupt endothelial cells. This phenomenon upregulates the acid sphingomyelinase (ASMase)-ceramide pathway and leads to endothelial cell apoptosis. Therefore, when a tumour is sensitized with microbubbles and subsequently treated with radiation, increased endothelial cell apoptosis is expected, which causes reduced microvascular density, and enhanced radiation-induced tumour cell death. Adapted from 'Focused Ultrasound and Ultrasound Stimulated Microbubbles in Radiotherapy Enhancement for Cancer Treatment,' by Leong KX, Sharma D, Czarnota GJ, 2023, Technol Cancer Res Treat, CC BY-NC [17], from 'Radiation enhancement using focussed ultrasound-stimulated microbubbles for breast cancer: A Phase 1 clinical trial', by Moore-Palhares D, et al., 2024, PLOS Medicine [19], and from 'A Novel Strategy to Improve Radiotherapy Effectiveness: First-in-Human MR-guided Focused Ultrasound- Stimulated Microbubbles (MRgFUS + MB) Radiation Enhancement Treatment,' by Moore-Palhares D, et al., 2023, Journal of Radiology and Oncology [22]. Abbreviations: ASMase, acid sphingomyelinase; FUS, Focussed ultrasound; MB, microbubbles; MR, Magnetic resonance; MRgFUS-MB, magnetic resonance-guided focussed ultrasound-stimulated microbubble treatment; US, Ultrasound. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

activated by shaking on a Vialmix unit (Lantheus Medical Imaging, USA) for 45 s, and then 1 mL of the product was injected intravenously, followed by a 10 ml flush of 0.9 % sodium chloride, before each treatment cell. The microbubble dose used per treatment cell is consistent with that used for clinical imaging [23] and reproduced our preclinical data [14]. Immediately after microbubble injection, FUS sequentially targeted each individual cell with a precise boundary of ≤60 μm. This was accomplished by employing a particular pulse sequence that involved a 16-cycle tone burst lasting 50 ms, followed by a delay period of 1950 ms before repeating the sequence [11]. This sequence was iterated over a duration of 5 min, resulting in a cumulative insonification time of 750 ms per treatment cell [11]. The individual treatment cells were sequentially activated using a step-and-shoot technique until the entire target tumour had been treated. For instance, if a tumour required 3 ultrasound treatment cells, the first cell was activated after administering 1 mL of microbubbles, followed by a pause of 30–60 s necessary for administration of an additional 1 mL of microbubbles, then immediate activation of the second cell, continuing until all cells were treated. The patients were then monitored for 30 min for safety reasons and subsequently transferred to the linear accelerator to undergo radiotherapy within two hours of completing MRgFUS-MB treatment. The choice of a 2-hour interval is supported by our previous research, which demonstrated synergistic effects when treatments were administered at intervals ranging from 0 to 12 h, while also considering logistics and patient convenience [12,13].

Outcomes

The primary outcome was the incidence of acute (≤3 months) adverse events graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 and was assessed for all patients who underwent at least one MRgFUS-MB treatment. Secondary outcomes were radiological response at 3 months for patients who completed a minimum 3-month follow-up, and local control (LC).

Patients underwent 1) clinical assessment on the days of MRgFUS-MB, and 2) clinical and radiological assessment with contrast-enhanced MR imaging at 1 week, 1 month, and 3 months after treatment completion. If patients were unable to undergo follow-up MR imaging for any reason, a contrast-enhanced computed tomography scan was performed as an alternative imaging modality. The stopping rules for premature trial suspension included the occurrence of 6 or more cases of acute grade ≥3 toxicity likely related to the MRgFUS-MB among the first 10 patients, or if any serious adverse event raised concerns regarding the safety of the intervention. Long-term clinical and

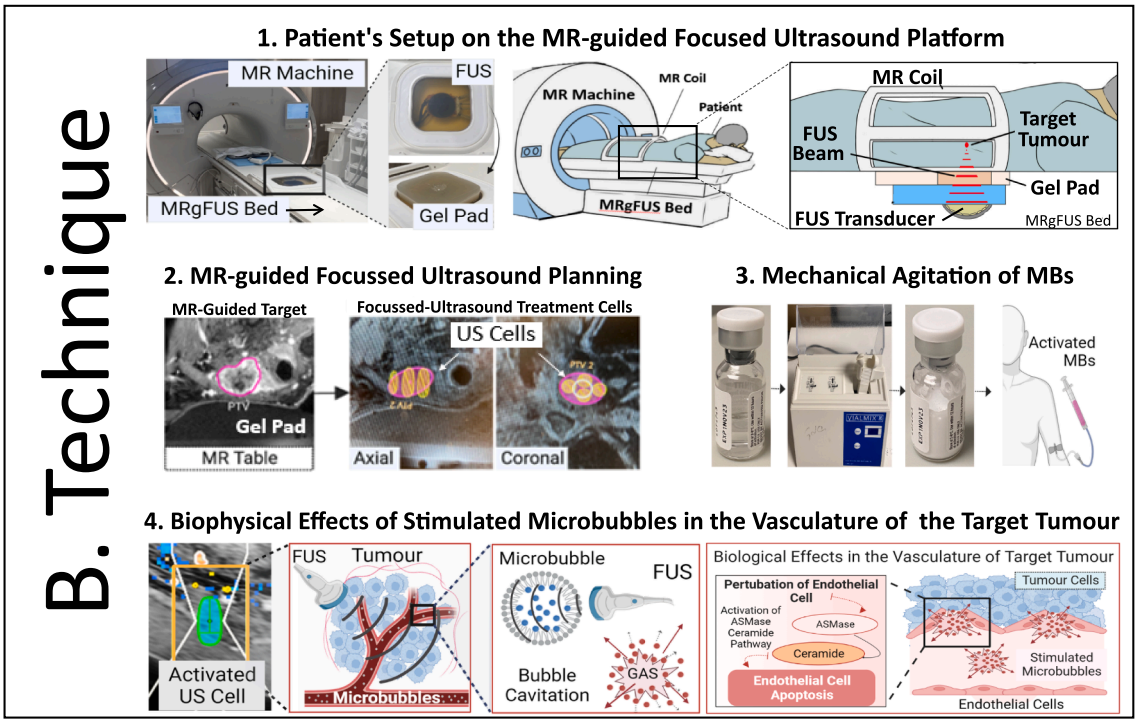

Fig. 1. (*continued*).

radiological follow-up (>3 months) was not pre-specified but was typically conducted every 3 months, or sooner if clinically indicated, as per the discretion of the treating radiation oncologist, following standard practice.

Response Evaluation Criteria in Solid Tumours V1.1 (RECIST) [24] was used to evaluate tumour response, with the tumour treated using MRgFUS-MB designated as the target lesion. The sum of the largest diameter of the target lesion was measured on follow-up imaging and compared to baseline: a reduction of >30 % indicated partial response, an increase of >20 % indicated progressive disease, and stable disease was determined if there was no significant decrease or increase in tumour size meeting the criteria for partial response or progressive disease. Complete response was defined by the disappearance of the contrast-enhancing tumour or, if targeting a lymph node, a reduction in the short axis to <10 mm [24].

Statistical analysis

Descriptive analyses were utilized to present pertinent patient characteristics. Clinical and demographical variables were presented as frequencies and percentages for categorical data (i.e., sex) and as median value accompanied by the range for continuous data (i.e., age). The duration from the start of MRgFUS-MB treatment to the occurrence of local progression was employed to compute LC. LC was determined utilizing the Kaplan-Meier method. Statistical analysis was conducted using the R software for Windows (version 2023.06.2 561 x64).

Results

Between June/2020 and January/2023, 12 patients were enrolled and allocated to intervention (Fig. 2). One patient withdrew consent after undergoing the first MRgFUS-MB treatment. No patients were lost to follow-up. A total of 11 patients were included in the safety and LC analyses. Three patients died due to advanced HN cancer (unrelated to the treated sites) before completing 3 months of follow-up and were not included in the 3-month tumour response assessment. The median follow-up was 7 months (range, 0.3–38).

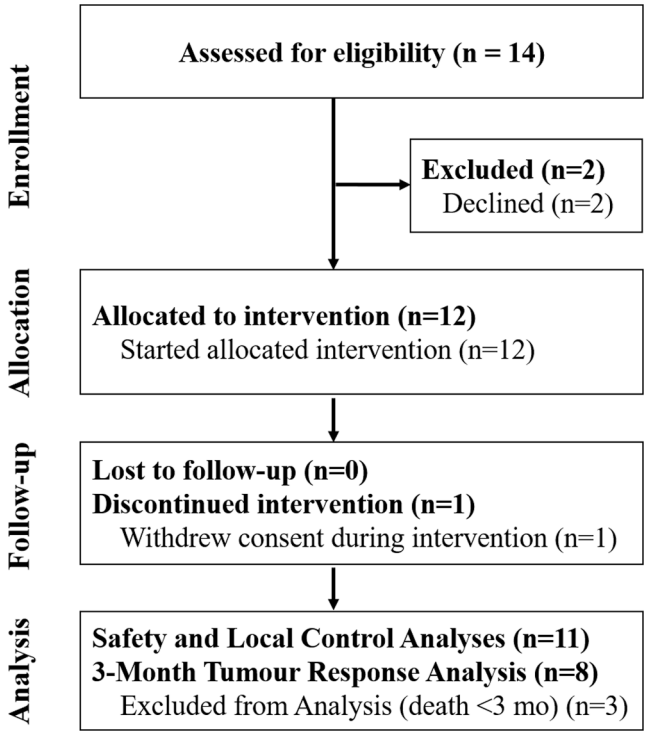

Fig. 2. CONSORT diagram illustrating the flow of participants through each stage of the clinical trial. Abbreviation: mo, months.

The characteristics of the included population were detailed in Table 1. The median age was 66 years (range, 57–90) and most patients were male (73 %, n = 8/11). All the tumours were squamous cell carcinomas. The majority of patients had oropharyngeal carcinoma (55 %, n = 6/11), T4N2-3 stage (55 %, n = 6/11), and non-metastatic disease (72 %, n = 8/11). Eight patients (72 %, n = 8/11) had *de novo* and four

Table 1
Patient, tumour, and treatment characteristics. Abbreviations: M, male; F, female; SCC, squamous cell carcinoma; RT, radiotherapy; MRgFUS-MB, magnetic resonance-guided focussed ultrasound-stimulated microbubble treatment; FUS, Focussed ultrasound; EOD, every other day; W, watts. *The 4 highlighted patients did not complete all the planned MRgFUS-MB sessions: 2 missed their final MRgFUS-MB session and the other 2 ended their last session prematurely before all the treatment cells were activated.

Patient	Age and Sex	Stage	Primary tumour	Treatment scenario	Histology and P16 status	Prior locoregional radiotherapy	Immediate prior systemic therapy	Current RT Regimen	Concurrent chemotherapy	MRgFUS-MB treatment			
										Target tumour	Number of sessions completed/ planned	Number of US cells completed/ planned per session	FUS power per session
#1	66y, M	T4N3M1	Larynx	Recurrent disease	SCC, P16-unknown	60 Gy/30 fractions	None	25 Gy/5 fractions (daily)	None	Left supraclavicular lymph node	2/2	1st = 5/5 2nd = 5/5	1st = 7 W 2nd = 7 W
#2	79y, M	T4N1M0	Maxillary	Recurrent disease	SCC, P16-unknown	50 Gy/20 fractions	None	20 Gy/5 fractions (daily)	None	Right maxillary sinus	2/2	1st = 3/3 2nd = 3/3	1st = 8 W 2nd = 8 W
#3	57y, M	T3N2M0	Oral cavity	Recurrent disease	SCC, P16-unknown	66 Gy/33 fractions	None	30 Gy/5 fractions (EOD)	None	Submental lymph node	2/2	1st = 4/4 2nd = 4/4	1st = 7 W 2nd = 7 W
#4	90y, M	T4N2M0	Oropharynx	De novo	SCC, P16-unknown	None	None	25 Gy/ 5 fractions (daily)	None	Base of tongue	2/2	1st = 5/5 2nd = 5/5	1st = 4 W 2nd = 4 W
#5	69y, M	T4N3M0	Hypopharynx	De novo	SCC, P16-negative	None	None	30 Gy/10 fractions (daily)	None	Right level II lymph node	2/2	1st = 4/4 **2nd = 3/4***	1st = 4 W 2nd = 4 W
#6	61y, M	T4N3M1	Oropharynx	De novo	SCC, P16-negative	None	None	30 Gy/10 fractions (daily)	None	Right level IX lymph node	**1/2***	1st = 6/6	1st = 4 W
#7	77y, F	T2N3M0	Oropharynx	De novo	SCC, P16-negative	None	None	70 Gy/ 35 fractions (daily)	None	Left level II lymph node	3/3	1st = 3/3 2nd = 2/2 3rd = 2/2	1st = 4 W 2nd = 4 W 3rd = 5 W
#8	65y, M	T4N2M1	Oropharynx	Recurrent disease	SCC, P16-negative	60 Gy/30 fractions	Carboplatin, paclitaxel and Pembrolizumab	25 Gy/5 fractions (daily)	None	Left level II lymph node	2/2	1st = 5/5 **2nd = 2/6***	1st = 6 W 2nd = 4 W
#9	58y, F	T2N3M0	Oropharynx	De novo	SCC, P16-negative	None	None	70 Gy/ 35 fractions (daily)	Cisplatin	Left level II lymph node	3/3	1st = 7/7 2nd = 6/6 3rd = 5/5	1st = 3 W 2nd = 4 W 3rd = 5 W
#10	70y, M	T3N2M0	Hypopharynx	De novo	SCC, P16-negative	None	None	70 Gy/ 35 fractions (daily)	None	Right level III lymph node	**2/3***	1st = 4/4 2nd = 5/5	1st = 5 W 2nd = 5 W
#11	66y, F	T3N2M0	Oropharynx	De novo	SCC, P16-negative	None	None	70 Gy/ 35 fractions (daily)	Cisplatin	Right level II/III lymph node	3/3	1st = 4/4 2nd = 5/5 3rd = 4/4	1st = 5 W 2nd = 5 W 3rd = 5 W

had recurrent disease (36 %, n = 4/11). Five patients were treated with 20–30 Gy/5 fractions (45 %, n = 5/11), 2 with 30 Gy/10 fractions (18 %, n = 2/11), and four with 70 Gy/30 fractions.

The majority of MRgFUS-MB treatments targeted bulky regional lymph nodes (82 %, n = 9/11). Patients received a median of 2 MRgFUS-MB treatments (range, 1–3), utilizing a median of 4 ultrasound treatment cells per session (range, 2–7). Four patients (36 %, n = 4/11) did not complete all planned radioenhancement treatments due to oncological pain or uncomfortable prolonged positioning. Among them, two patients (18 %, n = 2/11) missed their last MRgFUS-MB treatment, while the other two patients (18 %, n = 2/11) ended the session prematurely before all the treatment cells were treated (Table 1). The median duration of MRgFUS-MB treatment sessions was 70 min (range, 40–110).

Eleven patients were included in the safety analysis. All participants were assessed for toxicity during the MRgFUS-MB treatment; ten (91 %) were assessed at one week, nine (82 %) at one month, and eight (73 %) at three months after treatment. There were no instances of systemic adverse events resulting from microbubble administration during or after MRgFUS-MB treatment. The observed acute (≤3 months) adverse events were limited to the expected reactions following radiation treatment. The worst acute adverse event consisted of grade 3 toxicity in 6 patients (55 %, n = 6/11) and grade 2 in another 2 patients (18 %, n = 2/11). Acute adverse events included dermatitis in 7 patients (Grade 1 = 2, Grade 2 = 2, Grade 3 = 3), mucositis in 8 patients (Grade 1 = 5, Grade 2 = 2, Grade 3 = 1), and dysphagia in 9 patients (Grade 1 = 2, Grade 2 = 1, Grade 3 = 6), including 5 patients who already had grade 2–3 dysphagia at baseline. In the long term, no patients developed radiation necrosis or any other adverse event attributed to the MRgFUS-MB treatment. No grade 4 or 5 toxicities were observed.

Tumour response is detailed in Table 2 and Fig. 3. Eight patients completed the 3-month radiological follow-up and were included in the tumour response analysis. At 3 months, 4 patients (50 %, n = 4/8) achieved partial response, 3 had complete response (38 %, n = 3/8), and one patient developed progressive disease (13 %, n = 1/8). At the last follow-up, 4 patients (50 %, n = 4/8) had sustained complete responses (at 3, 14, 20, and 38 months), 1 patient (13 %, n = 1/8) had a partial response (at 5 months), and 3 exhibited progressive disease (38 %, n = 3/8). One case of exceptional response is represented by patient #3 (Tables 1 and 2, Fig. 3). This patient developed multiple locoregional recurrences which were further treated with the trial regimen here, followed by systemic therapy, as previously reported [22]. The target sites achieved complete response, systemic therapy was stopped, and since then, the patient has been on surveillance for over 3 years with no evidence of active disease. The LC rate at 3, 6, and 12 months was 88.9 % (95 % CI 70.6–100 %), 74.1 % (95 % CI 48.4–100 %), and 59.3 % (95 % CI 32.2–100 %), respectively (Fig. 4). Cases of sustained LC for over 20 months were exemplified by patients #3, 6, and 7 in Table 2 and Fig. 3.

Discussion

This study addresses the critical need for the development of selective radioenhancers. The treatment method here is supported by extensive preclinical research, which demonstrated that cavitation of intravascular microbubbles leads to vasculature disruption and the upregulation of pro-apoptotic pathways, ultimately increasing radiation-induced tumour cell death [8–12,25–27]. The current study is the first conducted in HN cancer patients and shows that this novel radioenhancement therapy is feasible, safe, and associated with encouraging tumour responses.

The safety of our treatment is evident from the absence of any systemic adverse events during or after microbubble administration, and notably, there were no cases of radiation necrosis or grade 4/5 toxicity. Additionally, the grade 3 toxicities observed (dermatitis, mucositis, dysphagia) were in line with what is typically expected following HN radiotherapy. Moreover, the safety and tolerability seen in the current study align with our previous publication of a similar phase I clinical trial that treated 18 breast cancer patients, and no relevant adverse events were observed [18,19].

The precise and selective effect of MRgFUS-MB treatment results from the ability of advanced three-dimensional FUS technology to precisely activate microbubbles exclusively within the target tumour area, with accuracy of 1 mm [28]. Consequently, potential side effects associated with the stimulated microbubbles and their intended bioeffects are expected to be confined to the target site, while microbubbles circulating elsewhere in the body remain inert and are cleared soon after treatment [29]. The systemic safety of the microbubbles utilized in our study (Definity, Lantheus Medical Imaging, USA) was well-documented in earlier publications [30,31], with an estimated 4.5 % risk of mild adverse events such as headache, nausea, or back pain [31], and less than 0.01 % risk of severe adverse events such as anaphylactic reactions [30].

We noted an encouraging objective response rate (partial and complete responses) of 88 % at the 3 months. Notably, this outcome was achieved despite all tumours being locally advanced, with the majority (64 %, n = 7/11) undergoing palliative courses of radiotherapy. It is worth highlighting that initially the treatment was administered to patients with notably extensive disease. However, as our department gained confidence in the treatment's safety and observed cases of exceptional and sustained complete responses (e.g., patient #3) [22],

Table 2
Response of the target tumour over time. Abbreviations: CR, complete response; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease. * The target LN had reduction in short axis to < 10 mm; therefore, it was classified as CR as per RECIST 1.1 criteria.

Patient	Short-term follow-up (≤3 months)					Long-term follow-up (>3 months)		Interval from treatment to progression
	Target tumour size at various time points (mm)				Tumour response at 3 months	Interval from treatment to last follow-up	Tumour response at last follow-up	
	Baseline	1 week	1 month	3 months				
#1	122 X 69	117 × 61	118 × 68	*NA*	*NA*	No long-term follow-up	–	No PD
#2	121 × 95	*NA*	*NA*	*NA*	*NA*	No long-term follow-up	–	No PD
#3	67 × 55	63 × 42	35 × 32	31 × 18	PR	38 months	CR	No PD
#4	43 × 36	36 × 35	*NA*	*NA*	*NA*	No long-term follow-up	–	No PD
#5	57 × 35	41 × 32	47 × 40	55 × 44	**PD**	10 months	**PD**	**3 months**
#6	45 × 26	27 × 16	13 × 9	0 × 0	CR	25 months	CR	No PD
#7	34 × 34	0 × 0	0 × 0	0 × 0	CR	20 months	CR	No PD
#8	51 × 45	32 × 19	25 × 19	22 × 20	PR	8 months	**PD**	**6 months**
#9	49 × 43	15 × 12	14 × 10	15 × 13	PR	14 months	**PD**	**8 months**
#10	38 × 31	*NA*	28 × 21	24 × 22	PR	11 months	PR	No PD
#11	24 × 22	*NA*	*NA*	9 × 7*	CR	3 months	CR	No PD

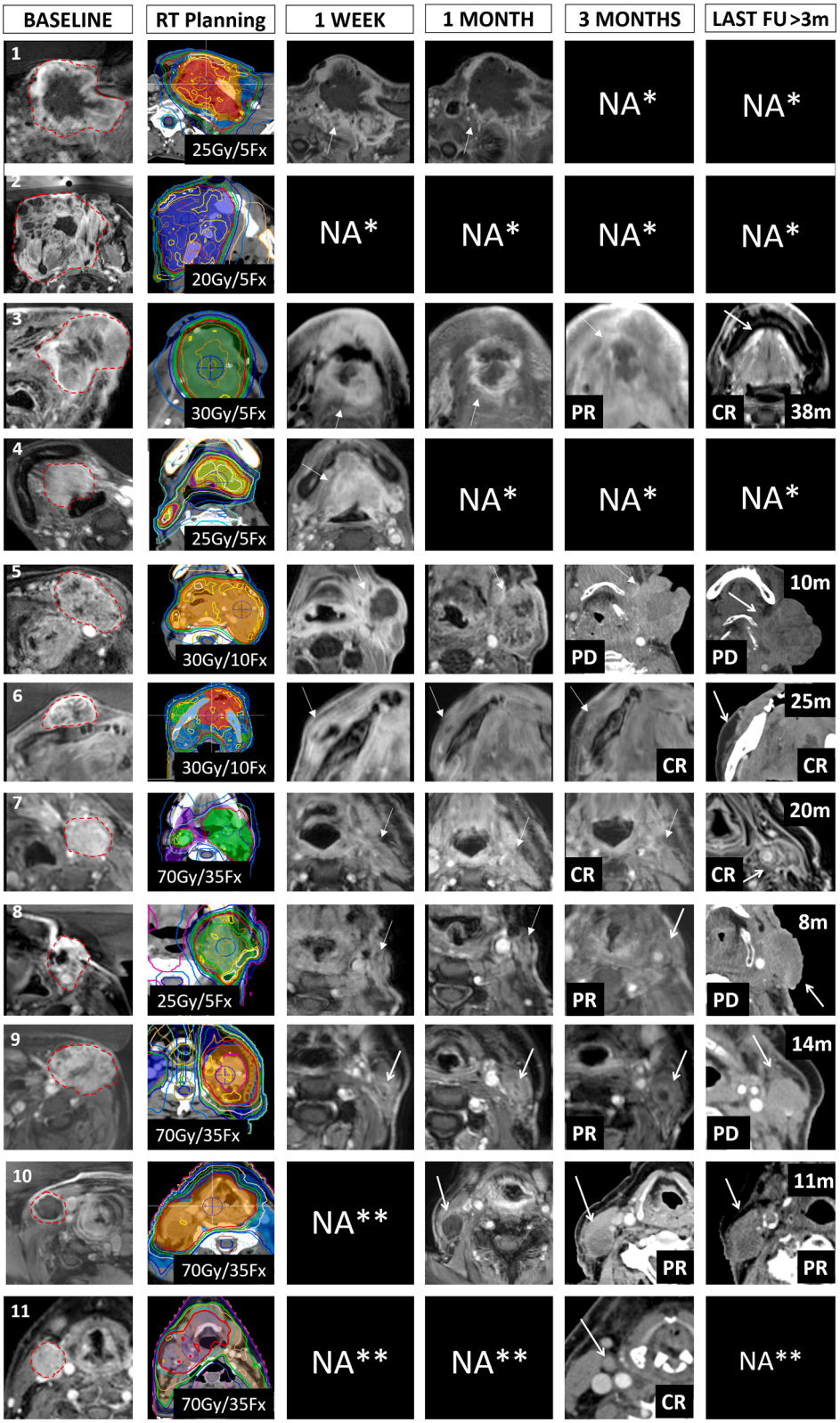

Fig. 3. Baseline imaging and radiological follow-up at different time points. The figure provides details of baseline and follow-up imaging for the MRgFUS-MB target tumour at various time points, along with the radiation treatment plan. In the baseline imaging, the dashed red line represents the target tumour. The prescription dose was detailed for each radiotherapy plan. White arrows indicate the treated tumour or the area where it was located in the case of a complete response during follow-up imaging. The numbers associated with the last follow-up indicate the time in months when the image was acquired from MRgFUS-MB treatment, for those who were followed for more than 3 months. At 3 months and the last follow-up, CR (complete response), PR (partial response), SD (stable disease), and PD (progressive disease) were used to denote the treatment response.* Patients died before reaching the specified follow-up interval. ** Patient did not undergo follow-up imaging at those time points. Abbreviations: NA, not available; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

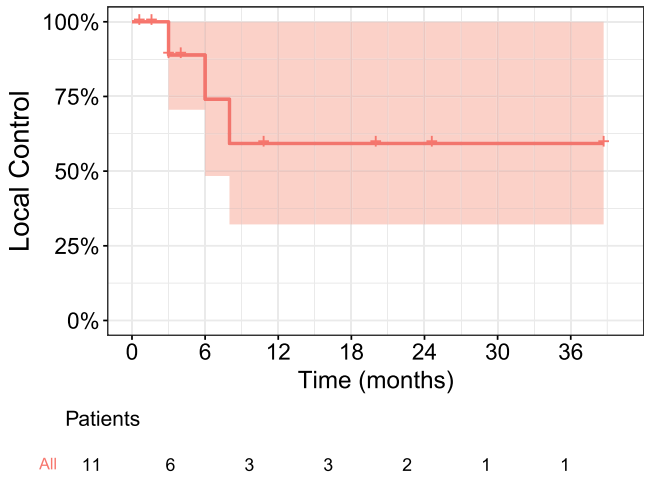

Fig. 4. Kaplan-Meier curve for local control of the tumours targeted by MRgFUS-MB. The solid line represents the probability of local control for the 11 target tumours. The shaded area represents the 95 % confidence interval around the estimated local control probability. Each step denotes an event of local failure and a short vertical line without a drop indicates the patients was censored.

oncologists became more confident in enrolling patients with less extensive disease and eventually extended it to those undergoing radical-intent treatment.

Due to the selectivity of MRgFUS-MB, we anticipate its potential to enhance tumour control without compromising toxicity, thereby improving the therapeutic index. Therefore, we hypothesized different plausible applications for HN cancer. Firstly, it could serve as a treatment-intensification strategy combined with concurrent chemo-radiation (70 Gy/35 fractions), particularly for less responsive tumours such as human papillomavirus (HPV)-negative [32,33]. Conversely, it could be employed for treatment de-intensification, especially in the case of HPV-positive tumours, which typically have a lower risk of local failure and more favourable prognoses [32,33]. In such cases, the radioenhancement treatment could be used to reduce radiation doses, thus mitigating radiation-induced toxicity while still maintaining the same chance of LC achievable with higher radiotherapy doses. These potential approaches warrant exploration through the development of clinical trials based on these hypotheses. Moreover, the ideal number of MRgFUS-MB treatments is unknown, and whether more frequent sessions (e.g., every 5 radiation fractions) provide better outcomes warrants investigation in future studies.

The synergistic interaction between FUS microbubbles and external beam radiation therapy is under investigation in a phase I study for melanoma/skin cancer (NCT05620290) and a recently launched phase II trial for breast cancer (NCT06185972). Taking a different approach, researchers at Thomas Jefferson University are conducting a Phase 2 clinical trial in which patients with hepatocellular carcinoma are randomized to receive transarterial radioembolization (TARE) either with or without ultrasound-stimulated microbubbles, and preliminary results are promising [34] (NCT03199274). Therefore, data from these clinical studies will play a crucial role in confirming the safety and efficacy of this radioenhancement treatment across diverse patient populations.

The main challenge encountered by patients undergoing MRgFUS-MB treatment in this study was positioning tolerability. Patients are required to remain immobile for an average of 70 min, and this could be difficult for HN cancer patients who often experience substantial baseline symptoms. As a consequence, despite the use of analgesics, four patients were unable to complete all planned ultrasound sessions. To overcome these challenges, the next generation of ultrasound therapy devices will activate all treatment cells simultaneously within a few minutes, eliminating the need for a step-by-step approach [35]. This advancement is expected to shorten treatment durations, significantly enhance positioning tolerability, and allow for an increase in the number of MRgFUS-MB sessions.

This study exhibits several strengths, primarily its innovative and translational nature, backed by compelling preclinical data supporting this approach [8–12,25–27]. Furthermore, patients underwent close assessments to ensure safety, and tumour response was meticulously evaluated using MR imaging, which is preferred due to its superior soft-tissue contrast. Nevertheless, our study does have limitations, including varying radiation regimens and target volumes. Moreover, although this study predominantly addresses the theory attributing the synergistic effect with radiation to the upregulation of the ASMase-ceramide pathway, it is essential to recognize the potential impact of other mechanisms triggered by ultrasound-stimulated microbubbles, such as transient increased tumour perfusion or the activation of alternative pathways for cell death signaling, which could independently contribute to the radioenhancement effect [21,36,37].

In conclusion, MRgFUS-MB treatment stands as a promising and innovative approach to enhance the effectiveness of radiotherapy for HN cancer. The treatment was feasible, well-tolerated, and associated with high rates of partial or complete responses at 3 months post-treatment. These findings warrant further investigation in a larger phase 2 clinical trial and highlight the potential use for other tumour histologies.

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CRediT authorship contribution statement

Daniel Moore-Palhares: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Murtuza Saifuddin:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Archya Dasgupta:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Maria Lourdes Anzola Pena:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Shopnil Prasla:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Ling Ho:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Lin Lu:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Joseph Kung:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Irene Karam:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Ian Poon:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Andrew Bayley:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Evan McNabb:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Greg Stanisz:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Michael Kolios:** Writing – review & editing, Methodology, Investigation. **Gregory J. Czarnota:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Irene Karam: Received honorarium for EMD Serono advisory board on locally advanced Head and neck cancers (Nov 2023).

Data availability

Data are stored in an institutional repository and will be made available on request to the corresponding author following institutional ethics committee protocols.

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