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CLINICAL INVESTIGATION

Randomized Phase 3 Trial of the Hypoxia Modifier Nimorazole Added to Radiation Therapy With Benefit Assessed in Hypoxic Head and Neck Cancers Determined Using a Gene Signature (NIMRAD)

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Purpose: Tumor hypoxia is an adverse prognostic factor in head and neck squamous cell carcinoma (HNSCC). We assessed whether patients with hypoxic HNSCC benefited from the addition of nimorazole to definitive intensity modulated radiation therapy (IMRT).

Methods and Materials: NIMRAD was a phase 3, multicenter, placebo-controlled, double-anonymized trial of patients with HNSCC unsuitable for concurrent platinum chemotherapy or cetuximab with definitive IMRT (NCT01950689). Patients were

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This trial is registered with the ISRCTN Registry and ClinicalTrials.gov and may be viewed online at https://classic.clinicaltrials.gov/ct2/show/NCT01950689.

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randomized 1:1 to receive IMRT (65 Gy in 30 fractions over 6 weeks) plus nimorazole (1.2 g/m^2 daily, before IMRT) or placebo. The primary endpoint was freedom from locoregional progression (FFLRP) in patients with hypoxic tumors, defined as greater than or equal to the median tumor hypoxia score of the first 50 patients analyzed (≥0.079), using a validated 26-gene signature. The planned sample size was 340 patients, allowing for signature generation in 85% and an assumed hazard ratio (HR) of 0.50 for nimorazole effectiveness in the hypoxic group and requiring 66 locoregional failures to have 80% power in a 2-tail log-rank test at the 5% significance level.

Results: Three hundred thirty-eight patients were randomized by 19 centers in the United Kingdom from May 2014 to May 2019, with a median follow-up of 3.1 years (95% CI, 2.9-3.4). Hypoxia scores were available for 286 (85%). The median patient age was 73 years (range, 44-88; IQR, 70-76). There were 36 (25.9%) locoregional failures in the hypoxic group, in which nimorazole + IMRT did not improve FFLRP (adjusted HR, 0.72; 95% CI, 0.36-1.44; $P = .35$) or overall survival (adjusted HR, 0.96; 95% CI, 0.53-1.72; $P = .88$) compared with placebo + IMRT. Similarly, nimorazole + IMRT did not improve FFLRP or overall survival in the whole population. In total (N = 338), 73% of patients allocated nimorazole adhered to the drug for ≥50% of IMRT fractions. Nimorazole + IMRT caused more acute nausea compared with placebo + IMRT (Common Terminology Criteria for Adverse Events version 4.0 G1+2: 56.6% vs 42.4%, G3: 10.1% vs 5.3%, respectively; $P < .05$).

Conclusions: Addition of the hypoxia modifier nimorazole to IMRT for locally advanced HNSCC in older and less fit patients did not improve locoregional control or survival.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy worldwide, with about 850,000 new cases and 450,000 deaths per year.[1] Forty percent of patients newly diagnosed with HNSCC are over the age of 65 years, a proportion that is expected to increase to 50% by 2040.[2] Older patients present at a more advanced stage of disease and, with the shared risk factors of smoking and/or excessive alcohol consumption, may have significant comorbidity.[3] Although combined modality therapies confer survival advantages in some patients with HNSCC, those who are older (>70 years) and/or less fit may not benefit from having concurrent chemotherapy[4] or cetuximab monoclonal antibody therapy[5] with radiation therapy. Despite the prevalence of HNSCC in older patients and inferior survival outcomes,[2,6] this population is underserved by clinical trials and treatment optimization remains a significant area of unmet clinical need.[7]

Tumor hypoxia is an adverse prognostic factor in HNSCC,[8] which induces a more aggressive cellular phenotype, promotes an immunosuppressive microenvironment, and reduces fixation of free radical–induced DNA damage.[9] A meta-analysis showed level 1a evidence in favor of adding hypoxic modification to radiation therapy in patients with HNSCC, an effect independent of the type of hypoxia modification.[10] Patients with more hypoxic HNSCC benefit most from hypoxia-modifying therapy.[11-13] Tumor hypoxia can be assessed in pretreatment tumor biopsies using a 26-gene signature upregulated under hypoxia.[14,15] The signature also predicted benefit from hypoxia-modifying treatment in laryngeal cancer, suggesting it could be used to personalize therapy.[16]

Nimorazole is a hypoxic radiosensitizer with high electron affinity, which enables the drug to mimic the effect of oxygen in rendering hypoxic cells radiosensitive. The DAHANCA 5 phase 3 study, including 422 patients with HNSCC, showed that nimorazole added to primary conventional radiation therapy (62-68 Gy, 2 Gy per fraction, 5 fractions per week) improved 5-year locoregional tumor control (49% vs 33%; $P < .002$), albeit with no significant improvement in overall survival (OS; 26% vs 16%; $P = .32$).[17] Since the 1990s, nimorazole use has been largely restricted to Danish practice.

We investigated whether addition of the hypoxia modifier nimorazole to intensity modulated radiation therapy (IMRT) improved outcomes for older and/or less fit patients with locally advanced HNSCC and more hypoxic tumors who were unsuitable for current standard-of-care concurrent platinum chemotherapy or cetuximab.

Methods and Materials

Study design

This was a multicenter, 2-arm, double-anonymized, placebo-controlled, randomized phase 3 trial done at 19 United Kingdom (UK) head and neck cancer treatment centers. The trial was coordinated by the Manchester Clinical Trials Unit. Sample collection, coordination, and analyses were done by the University of Manchester, UK. The first and last authors vouch for the accuracy and completeness of the data and analysis and for adherence to the study protocol. All authors contributed to the writing of the article. The study protocol is available online.

All centers and principal investigators completed the trial's central radiation therapy contouring and planning quality assurance accreditation. The approach was published,[18] with adherence centrally administered and monitored by the UK National Radiotherapy Trials Quality Assurance group and embedded in the study as a marker of quality for

a modern IMRT trial. Each center submitted contouring and planning benchmark cases for approval before recruiting patients. All centers were also required to submit at least their first oropharyngeal and hypopharyngeal cases for prospective contouring review and their first laryngeal case for retrospective review. Prospective planning review of the first 2 cases was required except where streamlining was approved by the independent Radiotherapy Trials Quality Assurance team because of prior participation in UK NIHR-portfolio phase 3 head and neck cancer IMRT trials.

Participants

The study was approved by the Research Ethics Committee of East of England Hatfield, UK (13/EE/0397). Patients were recruited by their treating clinicians and all patients provided written informed consent.

Eligible patients had newly diagnosed and histologically confirmed HNSCC of the oropharynx, hypopharynx, or larynx (American Joint Committee on Cancer [AJCC]/International Union for Cancer Control TNM seventh edition: T3N0-T4N0, T1N1-T4N3, or T2N0 base of tongue/hypopharynx). Patients were assessed as suitable for curative intent radiation therapy but unable to tolerate or unlikely to benefit from concurrent platinum chemotherapy or monoclonal antibody therapy by virtue of age, comorbidities, and/or performance status. Patients had an Eastern Cooperative Oncology Group/World Health Organization performance status (PS) score of 0 to 2, with adequate renal (creatinine $\leq 2 \times$ upper limit of normal [ULN]), hematological (absolute neutrophil count $\geq 1.5 \times 10^9$/L platelets $> 100 \times 10^9$/L), and hepatic function (total bilirubin $\leq 2 \times$ ULN, Aspartate transferase (AST) or Alanine transaminase (ALT) $< 3 \times$ ULN).

Formalin-fixed, paraffin embedded diagnostic tumor samples were prospectively collected. Human papillomavirus (HPV) and p16 status were determined centrally by a specialist head and neck pathologist. HPV status (positive or negative) was analyzed by in situ hybridization, and where unavailable p16 by immunohistochemistry was used as a surrogate (strong and diffuse nuclear and cytoplasmic staining in >70% of tumor was defined as positive); further details are included in Appendix E1.

For the hypoxia scores, RNA was extracted, reverse transcription performed, and the expression levels of the 26 signature genes analyzed using customized TaqMan Low-density Array microfluidic cards (Applied Biosystems); further details are included in Appendix E1. For patients with multiple blocks available, the block with the highest percentage of tumor tissue was used to determine the hypoxia score (HS). The median HS of the first 50 samples analyzed (0.079) was used as a prespecified value to define tumor samples as hypoxia-low (HS $<$ 0.079) or hypoxia-high (HS $\geq$ 0.079). The median HS was chosen as a previously validated cutoff to predict benefit of hypoxia modification.[16]

Randomization and masking

Eligible patients underwent computer-generated central randomization. Patients were randomly assigned in a 1:1 ratio to receive placebo plus radiation therapy or nimorazole plus radiation therapy. The method of randomization was permuted blocks within 16 strata defined by the 4 stratification factors. Only the study statistician, the main site hospital pharmacy (in providing the unmasking service), and the manufacturer were unmasked to allocations. Stratification factors were AJCC stage 2 and 3 versus stage 4, Eastern Cooperative Oncology Group/World Health Organization PS 0 and 1 versus 2, prior neck dissection, and use of a radiation therapy intermediate dose level. HPV status (positive vs negative) was an additional prespecified covariate for adjustment in the analysis because, at the inception of the trial, HPV status was not routinely available as standard of care at the time of randomization.

Procedures

Eligible patients were randomly assigned to have IMRT plus placebo or nimorazole. IMRT was given as a UK standard of 65 Gy in 30 once daily fractions of 2.17 Gy, 5 days a week, for 6 weeks. Placebo and nimorazole (1.2 g/m^2) were given as tablets (solid or dispersible preparation) taken orally (or via a feeding tube with an outer diameter of at least 4.0 mm) once a day, 5 days a week for 6 weeks, 90 minutes pre-IMRT. Adherence to the study drug was assessed by patient completed diaries.

NIMRAD used a geometric approach to define radiation clinical target volumes (CTVs). Before participation in NIMRAD, each trial center stipulated whether they would use 2 or 3 dose levels for all recruited patients. The dose for the radical planning target volume (PTV; 65 Gy), the intermediate PTV (60 Gy), and the elective PTV (54 Gy) were prescribed to the median of the respective volumes. For a 2-dose volume approach, CTV1 (65 Gy) included the gross primary or nodal gross tumor volume with a 1-cm isotropic margin and the whole of involved nodal level(s). CTV2 (54 Gy) included the remainder of the involved subsite and uninvolved nodal levels at risk of microscopic disease. For a 3-dose volume approach, CTV1 (65 Gy) included the gross primary or nodal disease with a 1-cm isotropic margin. CTV2 (60 Gy) included the remainder of the involved subsite and nodal level(s). CTV3 (54 Gy) included uninvolved nodal levels at risk of microscopic disease.

Response was assessed with a head and neck computed tomography (CT) or magnetic resonance imaging scan plus a chest CT or a whole-body fluorodeoxyglucose positron emission tomography−CT scan at 12 weeks after treatment. Patients were followed up at 6 and 12 weeks posttreatment, at 6, 12, 18, and 24 months, and then annually to 5 years.

Outcomes

The primary outcome measure was freedom from locoregional progression (FFLRP) from the time of randomization to a local or nodal failure in the hypoxia-high group. Treatment response was assessed 12 weeks after completion of radiation therapy. Persistent disease or subsequent disease recurrence at the primary site or neck were defined as locoregional progression events. Secondary outcomes for the hypoxia-high group and the whole population were FFLRP, OS as time from the date of randomization to death from any cause, cancer-specific survival as time from the date of randomization to death from head and neck cancer, disease-free survival as time from randomization to the first failure (local, nodal, or distant), or death from any cause. Common Terminology Criteria for Adverse Events version 4 (CTCAE v4) toxicities were assessed for the whole population at baseline, for acute effects weekly during treatment, 6 and 12 weeks posttreatment, and then for late effects at 6, 12, 24, and 36 months from treatment completion. Quality-of-life (The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ)-C30 and -Head and Neck 35 (H&N35), Hospital Anxiety and Depression Scale [HADs]) was assessed at baseline and after treatment at 6 weeks (for acute changes) and at 6, 12, 18, 24, and 36 months (for late changes).

Statistical analysis

NIMRAD was designed to investigate whether giving nimorazole with IMRT improved FFLRP with an assumed hazard ratio (HR) for benefit of 0.65, which was similar to that seen in the DAHANCA 5 trial.[17] With a time to the first locoregional failure endpoint and a 1:1 randomization, the trial required 470 patients to have 90% power in a 2-tail log-rank test at the 5% level of significance. In November 2018, it was apparent that the original target of 470 patients was not achievable with the available resource. On advice of the independent data monitoring committee, with no cross matching of hypoxia scores to outcome data and in order that the trial could still realize its objectives, the primary endpoint was amended to assess the difference in FFLRP in the hypoxia-high group. There was a revised target of 340 patients with recruitment not to extend beyond May 2019 We assumed an HR of nimorazole effect of 0.5 (with expected increased effectiveness of nimorazole in those with more hypoxic tumors),[13] that tumors would be broadly classified similarly by both the 26-gene and 15-gene signatures, tumor hypoxia status would be ascertained for 85% and there would be 66 locoregional failure events. The revised design had 80% power in a 2-tail log-rank test at the 5% level of significance. All outcome analyses were on an intention-to-treat basis. The primary locoregional progression outcome was analyzed within a Cox proportional hazards regression model with adjustment for prespecified covariates. If the proportionality assumption was not met, via an assessment of Schoenfeld residuals, then accelerated failure time models would be used. The primary outcome was a censored time-to-event variable measuring the time from randomization to locoregional failure. Censoring occurred where there was no preceding locoregional failure by the time of last follow-up or no preceding locoregional failure by the time of death. Planned secondary analysis included where death was considered as a competing risk using the Fine and Gray method.[19] Statistical significance was assessed at $P \leq .05$.

For CTCAE v4.0 recorded toxicities, a χ^2 test was performed to assess whether the proportions of the toxicity grades (G1+2, G3, G4) were significantly different between the nimorazole and placebo arms. Quality-of-life questionnaires were analyzed using a longitudinal ordinal or binary logistic regression model depending on the number of items in the question, with patient and time point as random effects and after adjusting for the prespecified covariates. Missing quality-of-life scores were not imputed. Statistical significance was assessed at $P \leq .05$. Individual question responses for the EORTC QLQ-C30 version 3.0 and H&N35 questionnaires were grouped. Scores were combined and normalized (from 0 to 100) as per the EORTC scoring manual, with comparisons made between nimorazole and placebo at each time point. A 10-point difference in scores was considered clinically relevant.[20] For the HADs questionnaire, mean anxiety and depression subscale scores and mean global scores were also compared between nimorazole and placebo at each time point. Analyses were done with R version 4.2.1

Results

Between May 2014 and May 2019, 338 patients were recruited and randomized (Fig. 1). There were 168 and 170 patients in the nimorazole and placebo arms, respectively. The HS-high group included 139 patients, with 70 and 69 in the nimorazole and placebo arms, respectively. The median follow-up time was 3.1 years (95% CI, 2.9-3.4). Patient and tumor characteristics are shown in Table 1 for the whole population and in Table 2 for the HS-high group; these were balanced between nimorazole and placebo arms (Appendix E2). Tumor HPV DNA status by in situ hybridization was available for 303 (90%), p16 by immunohistochemistry was used as a surrogate for 24 (8%), and 11 (3%) cases were unknown. Hypoxia scores were generated for 286 (85%) cases, with tumor samples received from 313 of 338 (93%) and sufficient RNA for downstream analysis obtained for 286 of 313 (91%). The median HS for the first 50 samples analyzed was 0.079, and for all samples was 0.077 (range, 0.02-0.36), with the frequency distribution included in Appendix E3.

For the HS-high group (HS $\geq$ 0.079), patients were mostly male (78%), the median age was 72 years (range, 45-88; IQR, 69-76), the PS was 2 for 16%, and there was a history of smoking for 91% (28% current smokers) and of

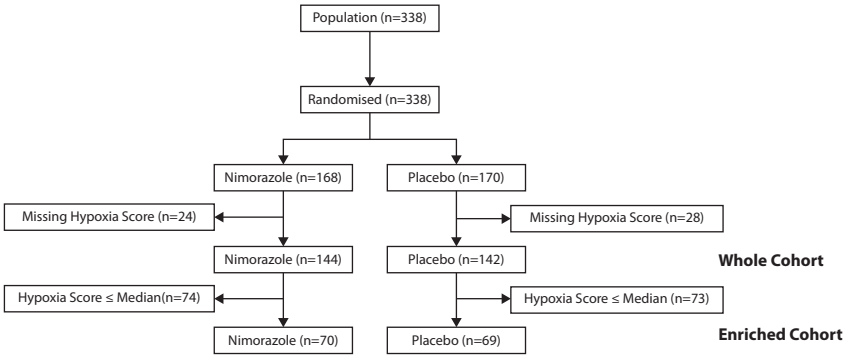

Fig. 1. Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

heavy alcohol intake for 34%. HNSCC tumor sites were oropharynx (51%), hypopharynx (21%), or larynx (28%). The AJCC seventh edition disease stages were II (4%), III (40%), and IVa/b (56%).

Overall, 137 (66%) of the oropharyngeal tumors were HPV-positive, and these accounted for 97% of the total number of HPV-positive cases (n = 142). The median HS for HPV-positive cases was 0.058 and for HPV-negative cases was 0.090. For HPV-positive tumors, 35% and 65% were in the HS-high and HS-low groups, respectively.

Radiation therapy adherence is shown in Table 3. Overall, 308 of 338 (91%) patients completed all 30 radiation therapy fractions, 147 of 168 (88%) and 161 of 170 (95%) for the nimorazole and placebo arms, respectively. In total, 10 (3%) patients did not commence radiation therapy and 20 (6%) stopped during treatment. Reasons for discontinuation included clinical deterioration, disease progression, patient choice, and study withdrawal. Overall, 67% and 73% of patients took nimorazole for ≥75% and ≥50% of radiation therapy fractions, respectively.

The primary endpoint of FFLRP in the HS-high group had 36 locoregional failure events, and although the direction of effect was in favor of nimorazole, there was no significant difference between nimorazole and placebo (adjusted HR, 0.72; 0.36-1.44; $P = .35$; Fig. 2a). For the whole population, there were 74 locoregional failure events and, similarly, there was no significant difference between nimorazole and placebo (adjusted HR, 0.76; 0.48-1.21; $P = .25$; Fig. 2b). The estimated 1- and 3-year locoregional control rates were 83% and 77% (1-year) and 77% and 72% (3-year) for the nimorazole and placebo arms, respectively. Where death was considered as a competing risk, there were similar findings for FFLRP (HS-high: subdistribution HR; adjusted HR, 0.64; 0.32-1.28; $P = .21$; whole population: HR, 0.73; 0.46-1.16; $P = .18$).

For the secondary endpoint of OS in the HS-high group and whole population, there were no differences between nimorazole and placebo arms (HS-high: adjusted HR, 0.96; 0.53-1.72; $P = .88$; Fig. 3a; whole population: HR, 0.92; 0.65-1.31; $P = .66$; Fig. 3b). The estimated 1- and 3-year OS rates were 82% and 80% (1-year) and 60% and 60% (3-year) for the nimorazole and placebo arms, respectively. For cancer-specific survival in the HS-high group and whole population, there were also no differences between the nimorazole and placebo arms (HS-high: adjusted HR, 0.89; 0.44-1.80; $P = .75$; Fig. 4a; whole population: HR, 0.92; 0.60-1.42; $P = .72$; Fig. 4b). For disease-free survival, in the HS-high group and whole population, there were no differences between nimorazole and placebo arms (HS-high: adjusted HR, 0.99; 0.60-1.64; $P = .98$; Appendix E4; whole population: HR, 1.00; 0.73-1.37; $P = .98$; Appendix E4). The numbers of outcome events for locoregional +/− metastatic failure, deaths +/− preceding failure are shown in Appendix E5.

Exploratory per-protocol analyses included evaluation in the whole population of nimorazole effect on FFLRP for those who completed all 30 fractions of radiation therapy (HR, 0.60; 0.26-1.37; $P = .23$; Appendix E6), for those who took nimorazole for >50% of radiation therapy fractions (HR, 0.60; 0.24-1.48; $P = .63$; Appendix E6), and for both (HR, 0.56; 0.21-1.46; $P = .23$). A 20-week landmark analysis for FFLRP, which excluded patients with treatment failure or censoring before 20 weeks from randomization (ie, before the posttreatment assessment scans done 12 weeks after completion of radiation therapy), showed similar HRs for the effect of nimorazole to those in the primary analyses and for the whole population (data not shown).

We also assessed whether the tumor HS status was prognostic for FFLRP in the whole cohort or for those with HPV-negative cancer after adjusting for the prespecified clinical variables in those treated with radiation therapy alone. There was no evidence of HS being prognostic in the whole cohort (HR, 1.01; 95% CI, 0.95-1.06; $P = .786$) or in the HPV-negative population (HR, 1.03; 95% CI, 0.97-1.10; $P = .388$). An assessment of nonlinearity between the hypoxia score and the primary endpoint was also conducted, which found no correlation. Subsequent exploratory analyses of different HS cutoffs did not predict benefit from the addition of nimorazole to radiation therapy. HPV status was prognostic for FFLRP and OS in the whole population (FFLRP: HR, 0.37; 0.21-0.63; $P < .001$; OS: HR, 0.60; 0.41-0.88; $P = .008$; Appendix E7). There was no effect of nimorazole on either FFLRP or OS for those with HPV-positive (FFLRP: HR, 0.69; 0.26-1.81; $P = .447$; OS: HR, 0.79; 0.43-1.47; $P = .461$) or HPV-negative cancer (FFLRP: HR, 0.76; 0.44-1.30; $P = .313$; OS: HR, 1.00; 0.64-1.54; $P = .985$; Appendix E7).

Table 1 Characteristics of the whole population

Variable	Level	Nimorazole	Placebo	Overall
Patients, no.		168	170	338
Trial arm (%)	Nimorazole	168 (100.0)	0 (0.0)	168 (49.7)
	Placebo	0 (0.0)	170 (100.0)	170 (50.3)
sex (%)	Male	133 (79.2)	129 (75.9)	262 (77.5)
	Female	35 (20.8)	41 (24.1)	76 (22.5)
Median age [Range] [IQR]		73 [44, 88] [70, 75]	73 [44, 88] [70, 76]	73 [45, 84] [70, 76]
Tumor site (%)	Oropharynx	110 (65.5)	97 (57.1)	207 (61.2)
	Hypopharynx	25 (14.9)	26 (15.3)	51 (15.1)
	Larynx	33 (19.6)	47 (27.6)	80 (23.7)
HPV status (%)	Negative	88 (52.4)	97 (57.1)	185 (54.7)
	Positive	75 (44.6)	67 (39.4)	142 (42.0)
	Missing	5 (3.0)	6 (3.5)	11 (3.3)
TNM version 7	II	7 (4.2)	8 (4.7)	15 (4.4)
stage (%)	III	52 (31.0)	51 (30.0)	103 (30.5)
	IVA	99 (58.9)	99 (58.2)	198 (58.6)
	IVB	10 (6.0)	12 (7.1)	22 (6.5)
WHO PS (%)	0	73 (43.5)	64 (37.6)	137 (40.5)
	1	69 (41.1)	80 (47.1)	149 (44.1)
	2	26 (15.5)	26 (15.3)	52 (15.4)
Neck dissection (%)	No	164 (97.6)	165 (97.1)	329 (97.3)
	Yes	4 (2.4)	5 (2.9)	9 (2.7)
Dose volumes (%)	2 doses (65/54 Gy)	86 (51.2)	87 (51.2)	173 (51.2)
	3 doses (65/60/54 Gy)	76 (45.2)	79 (46.5)	155 (45.9)
	Missing	6 (3.6)	4 (2.4)	10 (3.0)
Smoking status (%)	Never smoked	25 (14.9)	27 (15.9)	52 (15.4)
	Ex-smoker stopped for ≥1 y	80 (47.6)	87 (51.2)	167 (49.4)
	Ex-smoker stopped for <1 y	30 (17.9)	19 (11.2)	49 (14.5)
	Current smoker	32 (19.0)	37 (21.8)	69 (20.4)
	Missing	1 (0.6)	0 (0.0)	1 (0.3)
Alcohol intake (%)	Never heavy	121 (72.0)	111 (65.3)	232 (68.6)
	Ex-heavy	25 (14.9)	35 (20.6)	60 (17.8)
	Current heavy	22 (13.1)	24 (14.1)	46 (13.6)
Hypoxia score	No	24 (14.3)	28 (16.5)	52 (15.4)
obtained (%)	Yes	144 (85.7)	142 (83.5)	286 (84.6)
Hypoxia score Median [range] Mean [SD]		0.08 [0.02, 0.36] 0.09 [0.05]	0.08 [0.02, 0.30] 0.09 [0.06]	0.08 [0.02, 0.36] 0.09 [0.05]

Abbreviations: HPV = human papillomavirus; NEG = negative; POS = positive; PS = performance status; WHO = World Health Organization.

For CTCAE v4.0 toxicities (Appendix E8), nimorazole compared with placebo increased acute nausea up to 12 weeks from treatment (grade 1+2: 56.5% vs 42.4%; grade 3+4: 10.1% vs 5.9%; $P < .05$). There was increased late grade 1+2 hoarseness from 6 months after treatment with nimorazole (8.3% vs 6.5%; $P < .05$), but no other differences in grade 1+2 or higher toxicities. For quality-of-life, nimorazole caused no significant decrements in the EORTC QLQ-C30 functioning,

Table 2 Characteristics of the hypoxia-high group

Variable	Level	Nimorazole	Placebo	Overall
Patients, no.		70	69	139
Trial arm (%)	Nimorazole	70 (100.0)	0 (0.0)	70 (50.4)
	Placebo	0 (0.0)	69 (100.0)	69 (49.6)
sex (%)	Male	56 (80.0)	52 (75.4)	108 (77.7)
	Female	14 (20.0)	17 (24.6)	31 (22.3)
Median age [Range] [IQR]		72 [50, 88] [69, 75]	72 [45, 84] [66, 76]	72 [45, 88] [69, 76]
Tumor site (%)	Oropharynx	40 (57.1)	31 (44.9)	71 (51.1)
	Hypopharynx	14 (20.0)	15 (21.7)	29 (20.9)
	Larynx	16 (22.9)	23 (33.3)	39 (28.1)
HPV status (%)	Negative	46 (65.7)	51 (73.9)	97 (69.8)
	Positive	24 (34.3)	18 (26.1)	42 (30.2)
TNM version 7	II	3 (4.3)	2 (2.9)	5 (3.6)
stage (%)	III	29 (41.4)	27 (39.1)	56 (40.3)
	IVA	36 (51.4)	36 (52.2)	72 (51.8)
	IVB	2 (2.9)	4 (5.8)	6 (4.3)
WHO PS (%)	0	28 (40.0)	26 (37.7)	54 (38.8)
	1	30 (42.9)	33 (47.8)	63 (45.3)
	2	12 (17.1)	10 (14.5)	22 (15.8)
Neck dissection (%)	No	70 (100.0)	66 (95.7)	136 (97.8)
	Yes	0 (0.0)	3 (4.3)	3 (2.2)
Dose volumes (%)	2 doses (65/54 Gy)	39 (55.7)	41 (59.4)	80 (57.6)
	3 doses (65/60/54 Gy)	29 (41.4)	28 (40.6)	57 (41.0)
	Missing	2 (2.9)	0 (0.0)	2 (1.4)
Smoking status (%)	Never smoked	7 (10.0)	8 (11.6)	15 (10.8)
	Ex-smoker stopped for ≥1 y	30 (42.9)	30 (43.5)	60 (43.2)
	Ex-smoker stopped for <1 y	14 (20.0)	11 (15.9)	25 (18.0)
	Current smoker	19 (27.1)	20 (29.0)	39 (28.1)
Alcohol intake (%)	Never heavy	51 (72.9)	41 (59.4)	92 (66.2)
	Ex-heavy	10 (14.3)	20 (29.0)	30 (21.6)
	Current heavy	9 (12.9)	8 (11.6)	17 (12.2)
Hypoxia score obtained (%)	Yes	70 (100.0)	69 (100.0)	139 (100.0)
Hypoxia score Median [range] Mean [SD]		0.11 [0.08, 0.30] 0.13 [0.04]	0.12 [0.08, 0.36] 0.13 [0.06]	0.11 [0.08, 0.36] 0.13 [0.05]

Abbreviations: HPV = human papillomavirus; NEG = negative; POS = positive; PS = performance status; WHO = World Health Organization.

symptom, or global health scales. For some EORTC QLQ-H&N35 items, there were statistically significant differences between nimorazole and placebo (Appendix E9). However, none of the differences reached the minimal clinically important difference of 10 points (Appendix E10). For the HADs questionnaire, there were no significant differences overall for the anxiety or depression subscales or global scores between nimorazole and placebo (Appendix E11).

Discussion

The hypoxia modifier nimorazole added to IMRT did not improve locoregional tumor control or survival outcomes for locally advanced HNSCC in older and/or less fit patients who were unsuitable for concurrent platinum chemotherapy or cetuximab. This was seen in the overall population and in the enriched HS-high population.

Table 3 Radiation therapy adherence (whole population)

Variable	Level	Nimorazole	Placebo	Overall
Patients, no.		168	170	338
Delivered fractions (%)	0	6 (3.6)	4 (2.4)	10 (3.0)
	1-9	5 (3.0)	1 (0.6)	6 (1.8)
	10-19	7 (4.2)	3 (1.8)	10 (3.0)
	20-30	3 (1.8)	1 (0.6)	4 (1.2)
	30	147 (87.5)	161 (94.7)	308 (91.1)
Dose (Gy), median [Range]		65.0 [6.50, 66.0]	65.0 [6.50, 66.0]	65.0 [6.50, 66.0]
Treatment duration (d), median [Range]		39.00 [2.00, 50.00]	39.00 [2.00, 42.00]	39.00 [2.00, 50.00]

There were fewer locoregional failure events for the primary analysis than anticipated at study design from the results of the DAHANCA-5 trial, which recruited patients from 1986 to 1990.[17] This can be attributed to the change from conventional radiation therapy to IMRT, technical advances over time in IMRT delivery, introduction of centrally administered radiation therapy quality assurance,[21] and the increased incidence of HPV-positive HNSCC.[22] The direction of effect on locoregional tumor control favored nimorazole over placebo, but this was not statistically significant. It also did not translate to any difference in OS. This may be due to the high rates of intercurrent or treatment-related competing mortality events in this population.[23]

HPV-positive HNSCCs are a distinct tumor type, with different carcinogenesis,[24] increased radiosensitivity,[25] better treatment response, and improved prognosis.[26-28] HPV-positive compared with HPV-negative tumors had a lower median HS (0.058 vs 0.090), with most (65%) classified as HS-low. HPV-positive hypoxic cancers may also

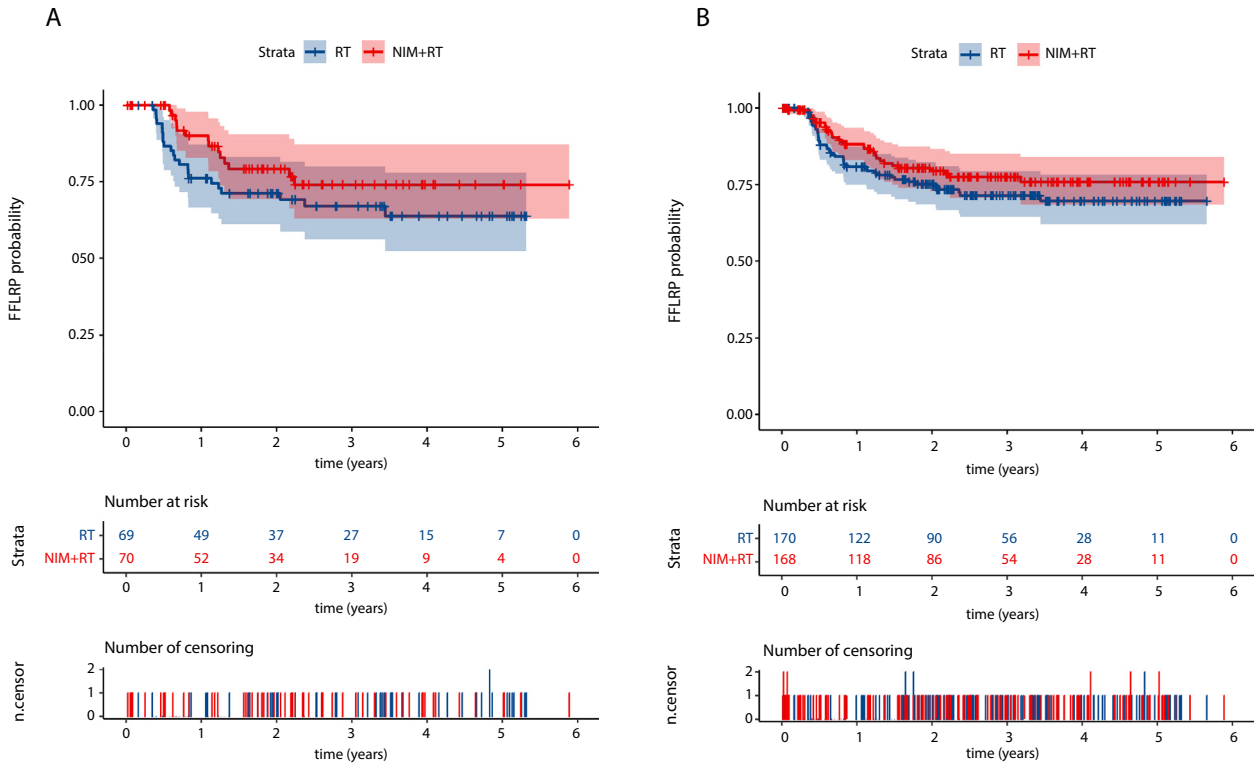

Fig. 2. (a) Freedom from locoregional progression for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the hypoxia-high group (adjusted hazard ratio, 0.72; 0.36-1.44; $P = .35$). (b) Freedom from locoregional progression for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the whole population (adjusted hazard ratio, 0.76; 0.48-1.21; $P = .25$).

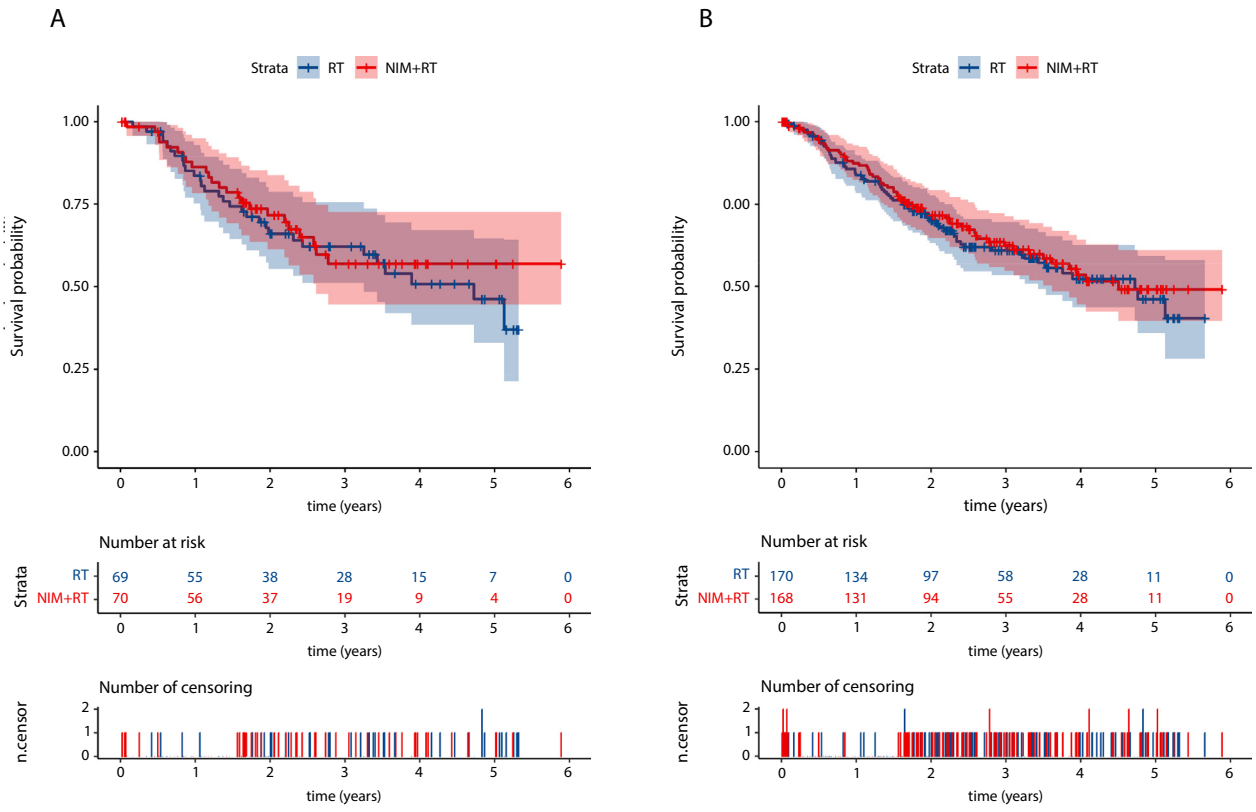

Fig. 3. (a) Overall survival for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the hypoxia-high group (adjusted hazard ratio, 0.96; 0.53-1.72; P = .88). (b) Overall survival for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the whole population (hazard ratio, 0.92; 0.65-1.31; P = .66).

not inherently benefit from hypoxia modification because of variation in the cellular, molecular, and immune responses to radiation.[9] In post hoc analysis of DAHANCA-5, for the 25% of patients with HPV-positive tumors (n = 84), there were similar locoregional control rates for those who took nimorazole or placebo (HR, 0.93; 95% CI, 0.45-1.91).[29] This finding was confirmed in NIMRAD, where there were similar locoregional control rates for the 42% of patients with HPV-positive disease. Further refinement of patient selection, such as by use of a hypoxia-immune classifier,[30] may identify those with HPV-positive and hypoxic HNSCC who benefit from nimorazole as a deintensification strategy.

HPV-negative HNSCC is associated with a higher rate of locoregional failure and poorer prognosis.[26] In later analysis of DAHANCA-5, a 15-gene hypoxia classifier predicted benefit from nimorazole in only HPV-negative tumors.[13] However, initial results of a randomized trial of nimorazole or placebo added to accelerated chemoradiotherapy in HPV-negative HNSCC that closed early with 194 of 640 patients recruited, showed no differences in 2-year locoregional control probability, either in the entire population (63.8% for nimorazole and 72.1% for placebo) or in those with hypoxia-high tumors, defined using the 15-gene classifier.[31] Post hoc analysis of NIMRAD did not show locoregional control benefit of nimorazole added to radiation therapy for HPV-negative HNSCC.

There was a moderate level of adherence to nimorazole, where 67% of patients took the drug for at least 75% of radiation therapy fractions. This is a similar finding to DAHANCA-5, where 62% took nimorazole for more than 75% of fractions.[17] Overall, 20 (6%) patients stopped radiation therapy before completion of the planned 30 fractions, of which 15 of 20 were in the nimorazole arm. However, nimorazole compared with placebo only caused more acute nausea, which was mainly low grade. Those unsuitable for concurrent chemotherapy can be a particularly challenging group of patients to study, given their diversity and lower tolerance for treatment, and there was an expected degree of noncompletion or compliance to the study protocol. We did exploratory analyses including those who completed all 30 radiation therapy fractions and more than 50% of nimorazole, which showed similar HRs for the effect of nimorazole to those in the primary analyses. There was no clinically meaningful detriment in long-term quality-of-life with nimorazole.

Patients were assessed as suitable for definitive radiation therapy but unable to tolerate or unlikely to benefit from concurrent platinum chemotherapy or monoclonal antibody therapy. NIMRAD included older patients (age

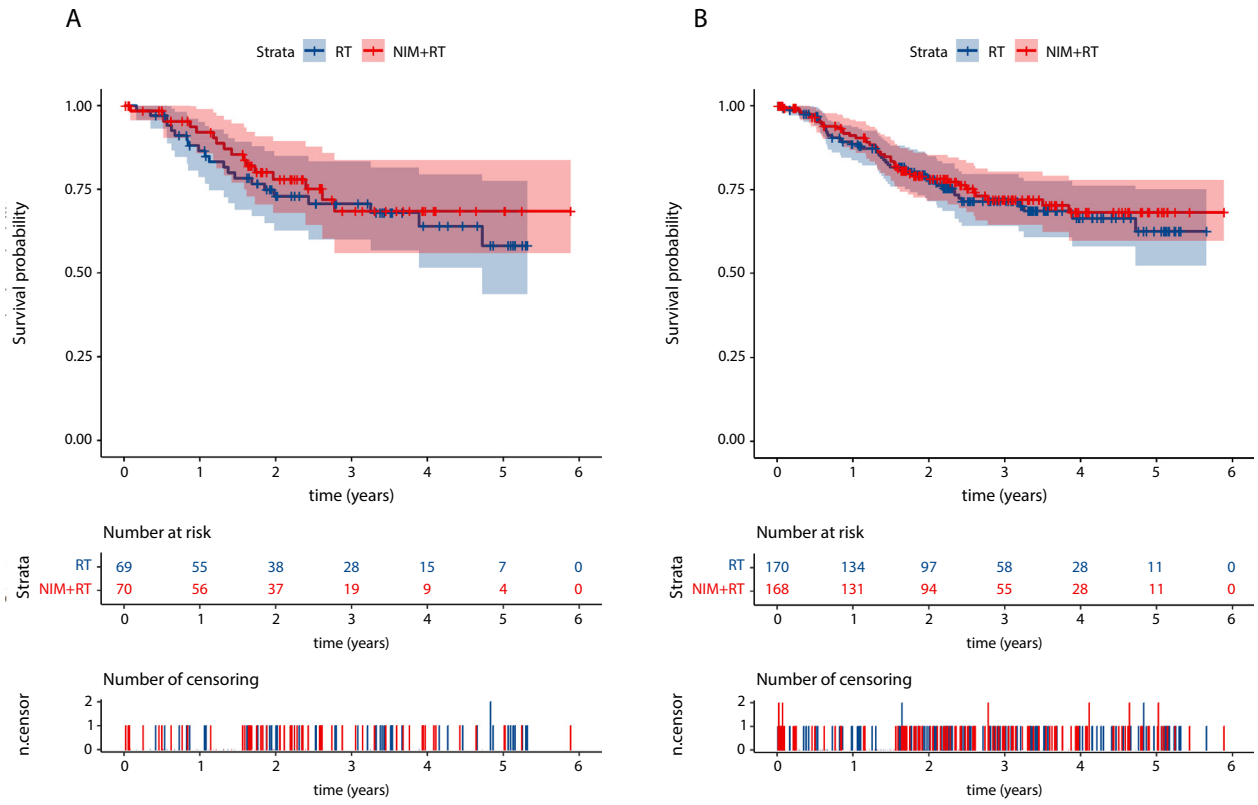

Fig. 4. (a) Cancer-specific survival for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the hypoxia-high group (adjusted hazard ratio, 0.89; 0.44-1.80; $P = .75$). (b) Cancer-specific survival for nimorazole plus radiation therapy compared with placebo plus radiation therapy in the whole population (hazard ratio, 0.92; 0.60-1.42; $P = .72$).

≥70 years for 75%) and those who were less fit on account of PS or comorbidities. The reasons individual patients were assessed as unsuitable for concurrent chemotherapy were not collected. The heterogeneity is a limitation of the trial, of relevance when considering generalizability of the results to routine practice. Although institutional and meta-analysis data question whether those who are older (eg, age >70 years) benefit from having concurrent chemotherapy[4] or cetuximab monoclonal antibody therapy,[5] the incorporation of frailty and comprehensive geriatric assessments supports and refines patient stratification for the use of concurrent systemic therapies beyond the application of a threshold age.[32]

There are few published randomized data in patients with locally advanced HNSCC who are ineligible for platinum chemotherapy. A recent phase 3 trial from Tata Memorial Hospital included 356 patients (age ≥70 years for 16%) treated in the definitive (61%) or adjuvant (39%) settings by conventional radiation therapy (80%) or IMRT (20%), with or without concurrent weekly docetaxel.[33] The trial showed a significant improvement in 2-year disease-free survival (42.0% vs 30.3%) and 2-year OS (50.8% vs 41.7%) for radiation therapy + docetaxel versus radiation therapy alone, respectively. Three other trials investigated IMRT plus immune checkpoint inhibitors compared with IMRT + cetuximab and did not show tumor control or survival benefit from the use of immunotherapy.[34-36]

The median HSs generated for NIMRAD samples were higher than those reported in a previous study.[16] Use of a population median cutoff was validated in several cohorts.[15,16] It was not possible to have a prestudy absolute cutoff as gene expression levels vary with age of sample, method of sample preparation, and platform used.[37] Although the type of sample (formalin-fixed, paraffin embedded) and platform (TaqMan Low-density Array) were the same as in a prior validation study,[16] sample age differed. RNA levels decline over time,[38] and the higher median HS can be attributed to higher RNA levels of the genes measured. We generated a median HS for the first 50 patients to have an absolute cutoff for validation within the trial and one that could subsequently be used prospectively in clinical practice if warranted.

HSs were not prognostic in the overall or HPV-negative cohort. A possible explanation is batch effects added noise to the data. Retrospective validation, such as that carried out by us,[16] involves cohorts where gene expression data were generated over a short period, in a single laboratory and by 1 or 2 operators. For NIMRAD, the quantitative polymerase chain reaction (qPCR) platform underwent prior technical validation,[14] good clinical laboratory practice procedures were used throughout, and only 2 operators were involved. However, samples were collected between 2014 and 2020 after patient enrolment and were analyzed in batches. Our

analytical platform was that used for the Oncotype DX assay —real-time quantitative polymerase chain reaction (RT-qPCR)—and is sensitive, specific, and highly reproducible. However, a paper published in 2018 showed quantitative PCR techniques can be unstable,[39] and the protracted period over which HSs were generated might have introduced uncertainty in clinical application.

There are several hypoxia-associated gene signatures, and it might be that others would perform better as a predictor of benefit from nimorazole, for example, the 15-gene classifier developed by the Aarhus group.[40] However, some studies showed the 2 signatures performed similarly in terms of hypoxia classification for locally advanced HNSCC,[41] prognostication in HPV-negative postoperative radiation therapy,[42] and TCGA[43] cohorts. Nevertheless, an agreement made before starting the trial involved sharing material so the 15-gene signature could be assessed independently, and RNA was sent to Denmark, with results awaited.

There are multiple approaches for assessing tumor hypoxia, with several shown to be both prognostic and predictive of benefit from hypoxia-modification of radiation therapy.[44] For example, an ^{18}F-FMISO positron emission tomography study confirmed its ability to be prognostic in patients with head and neck cancer and supported the concept of targeted dose escalation for the most hypoxic tumors.[45] The ^{18}F-MISO study closed early because of slow accrual attributed to the high complexity of the study setup for a multicenter trial. The pros and cons of the different methods are discussed elsewhere.[44]

Gene expression signatures are attractive because of the use of readily available diagnostic material, the expansion of approaches for measuring RNA expression, and the increasing use of signatures clinically. Our study was successful in carrying out a biomarker-driven multicenter radiation therapy trial highlighting feasibility. We assumed biomarker data (HSs) would be generated for 85% of patients, which was achieved. A limitation was that samples were not obtained for all patients (91%), but there was a high success rate in generating HSs from the samples received (93%).

Conclusion

NIMRAD did not show benefit of nimorazole added to radiation therapy in older or less fit patients with locally advanced HNSCC. As these groups are underserved by cancer clinical trials but account for over half of patients with HNSCC, the investigation of treatment optimization for this population continues to be an unmet need. The trial demonstrated success in generating hypoxia signature scores from 93% of tumor biopsies in a multicenter trial setting. The feasibility could have a bearing on future trial design of biomarker-driven trials, in which refining patient selection and the personalization of therapy based on molecular and genetic tumor characteristics remain important goals.

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