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Radiotherapy and Oncology 46 (1998) 135–146

RADIOTHERAPY & ONCOLOGY
JOURNAL OF THE EUROPEAN SOCIETY FOR THERAPEUTIC RADIOLOGY AND ONCOLOGY

A randomized double-blind phase III study of nimorazole as a hypoxic radiosensitizer of primary radiotherapy in supraglottic larynx and pharynx carcinoma. Results of the Danish Head and Neck Cancer Study (DAHANCA) Protocol 5-85

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Received 13 October 1997; revised version received 17 November 1997; accepted 2 December 1997

Abstract

Purpose: A multicenter randomized and balanced double-blind trial with the objective of assessing the efficacy and tolerance of nimorazole given as a hypoxic radiosensitizer in conjunction with primary radiotherapy of invasive carcinoma of the supraglottic larynx and pharynx.

Patients and treatment: Between January 1986 and September 1990, 422 patients (414 eligible) with pharynx and supraglottic larynx carcinoma were double-blind randomized to receive the hypoxic cell radiosensitizer nimorazole, or placebo, in association with conventional primary radiotherapy (62–68 Gy, 2 Gy per fraction, five fractions per week). The median observation time was 112 months.

Results: Univariate analysis showed that the outcome (5-year actuarial loco-regional tumor control) was significantly related to T-classification (T1–T2 48% versus T3–T4 36%, $P = 0.0008$), neck-nodes (N − 53% versus N + 33%), pre-irradiation hemoglobin (Hb) concentration (high 46% versus low 37%, $P = 0.02$) and sex (females 51% versus males 38%, $P = 0.03$). Overall the nimorazole group showed a significantly better loco-regional control rate than the placebo group (49 versus 33%, $P = 0.002$). A similar significant benefit of nimorazole was observed for the end-points of final loco-regional control (including surgical salvage) and cancer-related deaths (52 versus 41%, $P = 0.002$). This trend was also found in the overall survival but to a lesser, non-significant extent (26 versus 16%, 10-year actuarial values, $P = 0.32$). Cox multivariate regression analysis showed the most important prognostic parameters for loco-regional control to be positive neck nodes (relative risk 1.84 (1.38–2.45)), T3–T4 tumor (relative risk 1.65 (1.25–2.17)) and nimorazole (relative risk 0.69 (0.52–0.90)). The same parameters were also significantly related to the probability of dying from cancer. The compliance to radiotherapy was good and 98% of the patients received the planned dose. Late radiation-related morbidity was observed in 10% of the patients, irrespective of nimorazole treatment. Drug-related side-effects were minor and tolerable with transient nausea and vomiting being the most frequent complications.

Conclusion: Nimorazole significantly improves the effect of radiotherapeutic management of supraglottic and pharynx tumors and can be given without major side-effects. © 1998 Elsevier Science Ireland Ltd.

Keywords: Nimorazole; Radiotherapy; Head and neck carcinoma; Treatment-related morbidity; Hemoglobin; Randomized clinical trial

1. Introduction

Between 1979 and 1985, a randomized trial evaluating the hypoxic cell radiosensitizer, misonidazole, in the treatment of larynx and pharynx carcinoma (DAHANCA 2) was performed by the Danish Head and Neck Cancer Study Group [34]. Although no major overall significant benefit

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0167-8140/98/$19.00
PII S0167-8140(97)00220-X

was found, there was a significant improvement of locoregional control in the strata of patients with pharynx tumors. A similar tendency was observed in patients with supraglottic larynx carcinoma, whereas patients with glottic lesions did not show any benefit. Furthermore, the study revealed that patients with a low hemoglobin value had a significantly poorer prognosis if they belonged to the groups which also showed a benefit of misonidazole. The hemoglobin and misonidazole effects seemed to be independent and probably additive. Unfortunately, misonidazole induced significant peripheral neuropathy in 26% of the patients and it was concluded that it is unsuitable for further clinical use [34].

On this basis, it was decided to evaluate the less toxic hypoxic radiosensitizer, nimorazole (1-(*N*-*β*-ethyllmorpholine)-5-nitro-imidazole), in pharynx and supraglottic larynx tumors. This drug had previously been evaluated in preclinical and clinical phase I and II studies [38,39,47] and had so far not demonstrated any significant toxicity except transient nausea and vomiting. Although the hypoxic radiosensitizing ability is less than what theoretically can be achieved with misonidazole, the drug shows a flat dose–response curve implying that at clinically relevant doses, the hypoxic radiosensitizing ability is fairly high (approximately 1.3) [38]. Furthermore, the drug can be given in association with a conventional radiation therapy schedule and was therefore found to be suitable for use in the new study.

The observation from the DAHANCA 2 trial that a low hemoglobin value was associated with a poor local control was in agreement with numerous other studies [14,29,30]. A few small clinical trials have indicated that blood transfusions given to patients with low hemoglobin values may increase the tumor control probability in patients with carcinoma of the uterine cervix or the head and neck [5,14,29,44]. Therefore, this question was also addressed in the current study.

The present report has been performed according to the CONSORT guidelines for reporting clinical trials [2].

2. Patients and methods

2.1. Protocol design and patient eligibility

The Danish Head and Neck Cancer Study Protocol 5-85 was activated in January 1986 as a multicenter randomized and balanced double-blind trial with the objectives of assessing (i) the efficacy of nimorazole given as a hypoxic radiosensitizer in conjunction with radiotherapy of invasive carcinoma of the supraglottic larynx and pharynx, (ii) the tolerance and toxicity of nimorazole and (iii) the influence of hemoglobin concentration on tumor response to irradiation.

The trial was a double-blind study in which the control group received placebo instead of nimorazole. The study design, stratification and randomization arms are shown in Fig. 1.

The criteria for eligibility were invasive squamous cell carcinoma of the supraglottic larynx (stages 2–4, UICC 1982) or pharynx (stages 1–4), no evidence of distant metastases, normal liver and renal function and no neurological disorders expected to interfere with the drug treatment. The study was designed according to the Helsinki Declaration II and was approved by the local ethical committees. Prior to randomization the patients were stratified according to sex, institution, tumor site (supraglottic versus pharynx), tumor status (T1–T2 versus T3–T4) and hemoglobin concentration. The patients were then randomized to radiotherapy with nimorazole or placebo. Patients with low hemoglobin values were randomized to either receive or not receive a blood transfusion prior to inclusion in the nimorazole trial.

In patients where all eligibility criteria were fulfilled, patient data was entered into a local computer which generated the correct strata and randomization number and at the same time printed a confirmation letter which was sent to the data center. Each institution was supplied with a batch of consecutively numbered neutral looking sealed glasses. Each glass contained a total of 150 capsules with 500 mg nimorazole/placebo. The capsules were tasteless and were swallowed whole, not giving any indication of the presence of active drug. Each center was supplied with sealed envelopes indicating the randomization code. These envelopes were kept outside the radiotherapy department (at the hospital pharmacy) and could only be reached in the case of a clinical situation where knowledge of the presence of active drug was crucial for the further treatment of the patient. This did not happen in the present study and all envelopes were returned intact to the data center after completion of the trial. The trial has been maintained blinded during follow-up and the involved institutions are still unaware of which drug treatment the individual patients received. The trial profile and outcome are shown in Fig. 2.

2.2. Treatment

Radiotherapy was applied according to the DAHANCA

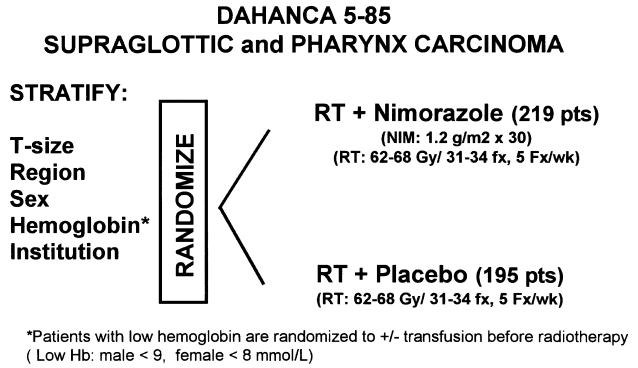

Fig. 1. Schematic representation of the trial design and randomization procedure for the Danish Head and Neck Cancer Study Protocol 5-85.

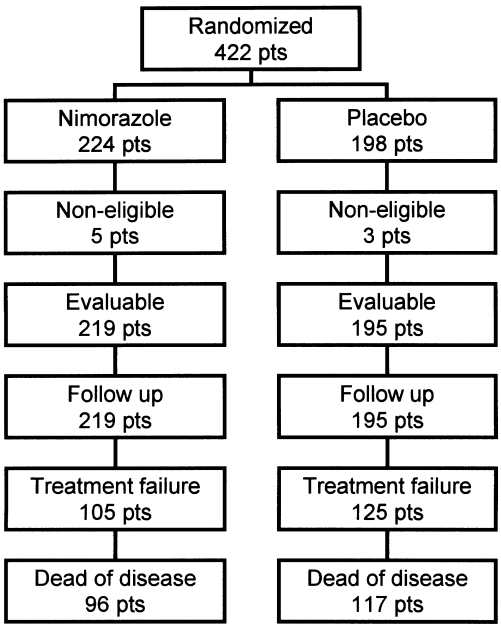

Fig. 2. Trial profile and outcome.

guidelines and given with 4–6 MeV photons or Co-60 using parallel opposed fields. Electrons were used to treat the neck in order to reduce the spinal cord dose, which was less than 50 Gy in all patients. The treatment was applied according to standardized fields including primary tumor and involved lymph nodes. A minimal tumor dose of 62–68 Gy (2 Gy per fraction, five fractions per week) was prescribed. The dose depended on the size of the tumor, with the larger tumors receiving the largest doses. Thus, patients with primary tumors and/or lymph nodes with a largest diameter of <2 cm were given 62 Gy, tumors between 2 and 4 cm were treated with 64 Gy and tumors above 4 cm were treated with 66–68 Gy. These doses were the minimal recommendations and a larger dose was allowed (Fig. 3). The fields included the first non-involved lymph node station, but after 50 Gy in 5 weeks the fields were reduced to include only the initially known macroscopic tumor. Table 1 gives the characteristics of radiotherapy treatment in the two randomization groups.

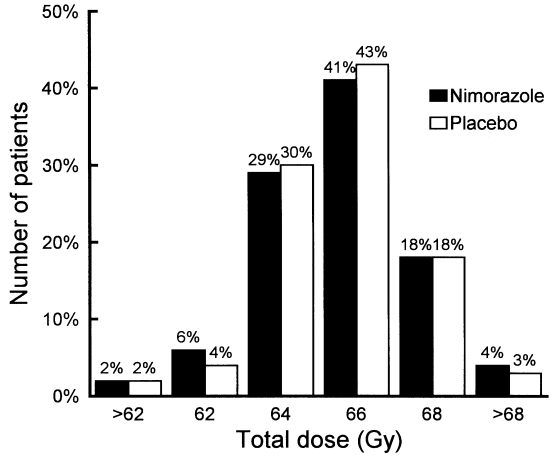

Fig. 3. Compliance to radiotherapy expressed as the total dose given as a function of the randomization group.

Table 1

Characteristics of radiation treatment in 414 evaluable patients as a function of the randomization group

	Radiotherapy alone (n = 195)	Radiotherapy + nimorazole (n = 219)
Total radiation dose (Gy)	66 (38[a]–72)	66 (24[a]–72)
Number of fractions	33 (19[a]–36)	33 (12[a]–38)
Overall treatment time (days)	50 (28[a]–85)	50 (19[a]–79)

All values given are the median (range).
[a]Includes 10 patients (five in each arm) who did not receive a dose above 60 Gy (see Fig. 3).

Nimorazole (or placebo) was obtained from Farmitalia, Milan. The dose and drug irradiation interval was based on prior pharmacokinetic toxicological evaluation [35,39,47]. The drug was administrated in the form of gelatine-coated capsules containing 500 mg active drug or placebo and was given orally 90 min prior to irradiation. The daily scheduled dose was approximately 1200 mg/m^2 body surface given in connection with the first 30 radiation treatment fractions. Patients with a surface of less than 1.6 m^2 received 1500 mg per day, those with a surface between 1.6 and 1.9 m^2 received 2000 mg per day and patients with a surface above 1.9 m^2 were given 2500 mg daily. The total dose was approximately 36 g/m^2 and was not allowed to exceed 40 g/m^2 or 75 g in total. Patients were instructed to take the capsules 90 min before radiation and the time was written on a schedule which was checked by the radiotherapy technician. Almost all radiation fractions were given with less than 15 min derivation from the planned 90 min.

Patients with low pre-irradiation hemoglobin (females <8 mmol/l; males <9 mmol/l) were randomized to receive or not to receive transfusion prior to final randomization to the hypoxic sensitizer. Transfusions were given with packed red blood cells to achieve a hemoglobin concentration in the 'high' value range. If during the treatment the hemoglobin level fell below the values indicated above, the transfusion was repeated. The hemoglobin level was measured every fortnight.

2.3. Assessments

Patients were followed at the oncological centers for at least 5 years or until death, with the exception of one patient who emigrated 29 months after treatment. The patients were evaluated with clinical examination weekly during treatment, 2 months after treatment, thereafter with 3-month intervals for the first year and with 4-month intervals for the second year and then twice annually for up to 5 years after randomization. Further examination was only performed if the patients had symptoms or evidence of recurrent disease. In addition to the recording of recurrence and/or survival status an attempt was made to record treatment-

related morbidity. Only patients with recurrence or other problems were subjected to regular follow-up for more than 5 years.

In the case of a residual tumor, recurrence or progression of the disease salvage surgery or palliative treatment was performed, depending on the status of the individual patient, symptoms and previous treatment.

2.4. Evaluation and statistical methods

All diagnostic, therapeutic and follow-up data were validated and processed by the DAHANCA data center. To optimize the data quality, the events recorded were cross-checked with the hospital records to ensure correct registration of the site or sites of failure and course of death.

The trial was designed to include 400 evaluable patients and was closed after that number had completed treatment. This number was estimated to be recruited over a 3-year period. Assuming a true improvement of the loco-regional tumor control rate from 40 to 55%, the probability that such an event would be detected at a significant level of $P < 0.05$ was greater than 90%.

The primary end-point was loco-regional control after radiotherapy. The definition of this end-point was complete and persistent disappearance of the disease in the primary tumor (T-site) and regional lymph nodes (N-site) after radiotherapy. The evaluation was performed clinically and supplemented with endoscopy and/or biopsy in case of doubt. Failure was recorded in the event of a recurrent tumor, or if the primary tumor never completely disappeared. In the latter situation the tumor was then assumed to have failed at the time of randomization. Since some uncertainty may exist concerning the time to an early recurrence, this end-point was not graphically presented until 6 months after randomization (Fig. 4). The primary end-point does not include the effect of a successful procedure with salvage surgery.

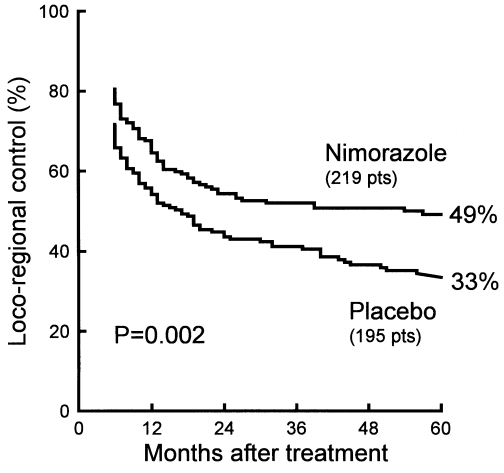

Fig. 4. Actuarial estimated loco-regional tumor control in patients randomized to receive nimorazole or placebo in conjunction with conventional radiotherapy for carcinoma of the pharynx and supraglottic larynx.

Secondary end-points include overall loco-regional control (including salvage procedures), disease-specific survival, overall survival and treatment-related morbidity. The end-point used for disease-specific survival is death from or with the actual cancer. The end-point for survival is any death, irrespective of cause. All time estimates were done using the date of randomization as the initial value.

Radiation-related morbidity was evaluated as graded reactions of acute mucositis and edema and late fibrosis, edema or necrosis, using the scoring system previously described [36].

The actuarial values of the end-points were evaluated by the Kaplan–Meier product-limit analysis using the BMDP 1L program. The Mantel–Cox test was used for comparison and a test for trend with equal weighing was performed if more than two groups were compared. The *P*-values estimated are those for a two-tailed test and the significance level was chosen to be 5%. Data are presented as 5-year actuarial values ± standard error of the mean, unless otherwise mentioned. Odds ratios with 95% confidence limits were calculated as described by Stell [45].

A multivariate Cox proportional hazards analysis was used to evaluate prognostic parameters and treatment with respect to the risk of loco-regional failure and disease-specific death, using the BMDP 2L program (version 7.0). Parameters were included in the model using forward selection and statistical analysis was performed by the Wald test.

The treatment effect was evaluated using 'the intention to treat' principle and patients were included in their randomization group irrespective of whether or not they had completed the planned treatment. The time for evaluation of loco-regional recurrence and disease-specific survival was 5 years after randomization, since patients were only followed regularly for that period. However, the date for evaluation of overall survival was 31 August 1997, which gave a median observation time of 112 months for that end-point (range 84–140 months).

3. Results

From January 1986 to September 1990, 422 patients were included. The accrual rate was constant throughout the period. Of the 422 included patients, eight were not eligible for the protocol. One had distant metastasis at the time of diagnosis, five were supraglottic T1,N0 (stage 1), one had incorrect histology (carcinoid carcinoma) and one had received surgery (neck dissection) prior to radiotherapy. Thus, 414 patients were evaluable for analysis and will be described below (Fig. 2).

There were 110 females and 304 males with a median age at randomization of 60 years (range 21–84 years). Distribution as a function of the stratification group followed the expected pattern (Table 2) and with the exception of more N3 patients in the placebo arm, no significant differences were found between the nimorazole and placebo groups.

Table 2
Characteristics of 414 evaluable patients and tumors as a function of the randomization group

	Radiotherapy alone	Radiotherapy + nimorazole
All patients	195	219
Age (years)		
Median	60	60
Range	24–84	21–84
Sex		
Female	49	62
Male	146	157
Tumor site		
Supraglottic	57	68
Oropharynx	91	96
Hypopharynx	25	28
Rhinopharynx	22	27
T-classification		
T1	30	36
T2	62	69
T3	62	72
T4	41	42
N-classification		
N0	79	108
N1	52	53
N2	8	19
N3	56	39
Staging (UICC 1978)		
Stage 1	5	16
Stage 2	34	36
Stage 3	67	85
Stage 4	89	82
Histopathological differentiation		
Well	20	24
Medium	55	60
Poor/undifferentiated	67	84
Not determined	53	51
Hemoglobin		
High	116	127
Low[a]	79	92
Transfused	34	48
Not transfused	45	44

[a]Females <8, males <9 mmol/l.

However, there were more patients randomized to the nimorazole arm due to an imbalance in randomization which by chance was caused by the large number of stratification groups. A total of 219 patients were randomized to receive irradiation plus nimorazole and a total of 195 patients were randomized to receive irradiation plus placebo.

At the time of evaluation, 229 patients had failed to achieve persistent loco-regional control within the irradiated volume. A total of 213 patients had died with or of the actual disease and overall 307 patients had died. All patients alive at the time of analysis were free from disease.

The use of nimorazole significantly improved the outcome (Table 3), with univariate odds ratios of 1.97 (95% CI 1.33–2.93) for loco-regional control and 1.92 (95% CI 1.30–2.84) for disease-specific survival and with a non-significant trend towards improvement in the overall survival with an odds ratio of 1.32 (95% CI 0.84–2.05). In contrast, the risk of development of radiation-related severe late morbidity was not influenced by nimorazole.

The primary loco-regional tumor control after radiotherapy is analyzed in Table 4 and Fig. 4. A statistically significant improvement in loco-regional tumor control was found in nimorazole-treated patients compared to patients receiving radiotherapy and placebo (5-year actuarial rate of 49 versus 33%, $P < 0.002$). This difference was observed in both the primary tumor and neck node response. Thus, the difference in 5-year actuarial values for T-site control was 57 versus 40% ($P < 0.004$) for the nimorazole- and placebo-treated groups, respectively. For neck node control the corresponding values were 71 versus 59% ($P < 0.04$), respectively.

The outcome also confirms the major prognostic factors in head and neck cancer (Table 4). Based on the previous DAHANCA 2 study, the stratification parameters in the DAHANCA 5 protocol were chosen to select equally balanced groups with distinct prognostic parameters. Univariate analysis (5-year actuarial loco-regional tumor control) showed a prognostic influence of T-classification (T1–T2 48% versus T3–T4 36%, $P = 0.0008$), pre-treatment hemoglobin (high 46% versus low 37%, $P = 0.02$) and sex (females 51% versus males 38%, $P = 0.03$). On the other hand, patients with supraglottic larynx cancers did not seem to differ from patients with pharynx tumors (Table 4), but it should be remembered that stage 1 supraglottic patients were not included in the trial.

The beneficial effect of nimorazole was present in most of the subgroups analyzed (Table 5), suggesting that the effect of the hypoxic sensitizer was not limited to certain subpopulations or tumor types.

Salvage surgery was successfully performed in 29 patients with failure in the T- or N-site. The final loco-regional control was therefore slightly better than after radiotherapy (Table 3), but since salvage procedures were at least as successful in the nimorazole-treated patients, the final loco-regional tumor control (including salvage) was also significantly better in the nimorazole group. In a similar way nimorazole treatment also resulted in a significantly higher cure rate with organ (i.e. larynx) preservation (Table 3).

Analysis of the failure pattern after treatment showed that the large majority of treatment failure was due to insufficient loco-regional tumor control. As a consequence the disease-specific survival was strongly related to insufficient loco-regional treatment and therefore also significantly better in patients given nimorazole (Fig. 5). This trend was also found in the overall survival but to a lesser, non-significant extent with 10-year actuarial survival rates of 26 and 16% ($P = 0.32$) for patients given nimorazole and placebo, respectively. This smaller difference in overall survival was probably due to a high incidence of death from other causes in this kind of patient.

In a Cox multivariate regression analysis, positive neck

Table 3
Overall status of primary and secondary end-points

	No. of patients	Total no. of patients	Odds ratio (95% CI)[a]
Loco-regional control (primary)			
Nimorazole	115	219	1.97 (1.33–2.93)
Placebo	70	195	
All	185	414	
Loco-regional control (salvage)			
Nimorazole	131	219	2.05 (1.35–2.95)
Placebo	82	195	
All	213	414	
Organ preservation			
Nimorazole	119	219	1.99 (1.34–2.95)
Placebo	73	195	
All	192	414	
Disease-specific survival			
Nimorazole	123	219	1.92 (1.30–2.84)
Placebo	78	195	
All	201	414	
Overall survival			
Nimorazole	62	219	1.32 (0.84–2.05)
Placebo	45	195	
All	107	414	
Severe late radiation morbidity			
Nimorazole	9	150	0.71 (0.24–2.04)
Placebo	6	139	
All	15	289	

[a]An odds ratio above unity indicates a benefit in the nimorazole-treated group.

nodes, advanced T-classification and absence of nimorazole treatment turned out to be the independent significant prognostic parameters using time to loco-regional failure as the end-point. The same parameters, with the addition of male sex, were also significant independent prognostic indicators with regard to the probability of dying from cancer (Table 6).

3.1. Hemoglobin and transfusion

A total of 171 patients had low hemoglobin values, as defined above. Among these, 82 were randomized to receive transfusion with packed red blood cells prior to the start of radiotherapy. Six patients did not receive transfusion, either because the hemoglobin value in a subsequent measurement was above the required value or due to incompliance with the protocol. The remaining patients received between 1 and 6 units of blood, but only 29 reached and maintained a hemoglobin level above the target value.

The expected prognostic effect of high and low hemoglobin values was demonstrated in univariate analyses (Table 4). This significant difference (46 versus 37%) was observed despite the fact that almost half of the low hemoglobin patients had received a blood transfusion. However, transfusion of patients with low hemoglobin concentrations did not significantly improve the outcome (Table 4). The number of patients submitted to this subrandomization was, however, too small to reach definitive conclusions and at the time of closure of the present protocol, it was therefore decided to continue to address this problem in the subsequent DAHANCA 7 study. Thus, further analysis of the influence of transfusion on the outcome of radiotherapy in head and neck cancer in patients treated with or without additional hypoxic radiosensitization with nimorazole awaits an overall analysis of the two studies.

The relationship between tumor response, nimorazole and hemoglobin concentration is seen in Table 5. This table shows that both a high hemoglobin concentration and the presence of nimorazole were good prognostic parameters and that nimorazole apparently sensitizes patients with both high and low hemoglobin values.

3.2. Radiation-related compliance and morbidity

Both radiotherapy and drug treatment were relatively well tolerated. Compliance with radiotherapy was the same in both treatment groups and only 10 patients did not complete the scheduled radiation treatment (five in each randomization group), due to deterioration in the general condition or death during treatment. Thus 98% of the patients completed the planned radiotherapy (Fig. 3).

The acute radiation-related morbidity was the same in the two randomization groups, with an incidence of moderate to severe mucositis of 60 and 62% in patients treated with nimorazole and placebo, respectively. For the same groups the incidences of severe acute edema were 10 and 9%, respectively.

Late radiation-related morbidity could be recorded in 289

Table 4
Univariate analysis[a]

	No. of patients	Loco-regional control (%)	*P*-value
All	414	42 ± 3	
Hypoxic sensitizer			
Nimorazole	219	49 ± 4	0.002
Placebo	195	33 ± 4	
Age (years)			
≤60	208	43 ± 4	0.33
>60	206	41 ± 4	
Sex			
Female	110	51 ± 5	0.03
Male	304	38 ± 3	
Tumor site			
Supraglottic	125	45 ± 5	0.31
Pharynx	289	41 ± 3	
T-classification			
T1	66	54 ± 7	0.0001
T2	131	46 ± 5	
T3	134	40 ± 5	
T4	83	30 ± 5	
N-classification			
N0	187	53 ± 4	<0.0001
N1	105	40 ± 5	
N2	27	37 ± 9	
N3	95	23 ± 5	
Staging (UICC 1978)			
Stage 1	21	60 ± 11	<0.0001
Stage 2	70	55 ± 6	
Stage 3	152	48 ± 4	
Stage 4	171	29 ± 4	
Differentiation			
Well	44	39 ± 8	0.24
Moderate	115	37 ± 5	
Poor/undifferentiated	151	46 ± 4	
Not determined	104	44 ± 4	
Hemoglobin			
High[b]	243	46 ± 3	0.02
Low	171	37 ± 4	
Transfused	82	39 ± 6	0.86
Not transfused	89	35 ± 6	

[a]5-year actuarial value of loco-regional tumor control.
[b]Females <8, males <9 mmol/l.

patients given sufficient follow-up time. The 5-year actuarial probability of developing severe late radiation-related complications (mainly in the form of late fibrosis, edema or necrosis) was 10 ± 4% in the nimorazole group and 9 ± 3% for patients given placebo ($P = 0.54$). This implies that nimorazole did not significantly influence the radiation-related side-effects.

3.3. Drug tolerance and toxicity

The tolerance and compliance to nimorazole/placebo is shown in Table 7. Compliance with drug treatment was dependent on whether the patient received nimorazole or placebo, especially due to the increased gastrointestinal toxicity in the nimorazole group. As a whole, 60% of the patients achieved the planned drug treatment (nimorazole 51%, placebo 70%) and 70% achieved more than 25 drug treatments (nimorazole 62%, placebo 79%). The cause of failure to fulfil the treatment was due to acute toxicity, as well as patients refusing or forgetting to take the drug or to difficulties in swallowing the large capsules (nimorazole 16%, placebo 16%). Only in 22% of the nimorazole patients and in 6% of the placebo patients was the treatment ceased due to direct toxicity.

Most patients completed the sensitizer treatment without any notable symptoms, whereas nausea and vomiting were the major complaints in the remaining group. In addition, flushing and dizziness were seen in a number of patients as well as transient cases of skin rash. A few cases of mild neurological symptoms were described, but a direct relationship to drug therapy is doubtful, as they occurred in patients with a history of chronic alcohol abuse.

A total of 35% of the patients receiving nimorazole treatment showed some form of side-effects, however, none of these have been severe or persistent and as a whole nimorazole has demonstrated a lack of important toxicity. No relationship between sex or the given drug dose has been found. In addition 12% of the placebo patients indicated side-effects. Thus, the toxicity has so far been acceptable and has been expressed as acute and reversible changes which have caused no major discomfort to patients.

3.4. Plasma nimorazole

Routine pharmacokinetic analysis was performed in 166 patients during the initial treatment with nimorazole. Multiple plasma samples were drawn during the first 6–8 h in order to get information about the pharmacokinetic properties of the drug. In order not to reveal the treatment code blood samples were drawn from both nimorazole- and placebo-treated patients. The plasma concentrations were measured on HPLC as previously described [38,39].

The peak plasma concentration ranged from 14 to 84 mg/l with a median value of 32 mg/l. In most patients (67%) the peak plasma concentration is achieved within 90 min after intake of the capsules but a considerable variation in absorption times has been found. A significant decrease in peak plasma concentration with increasing peak time was observed [35]. A total of 86% of the patients obtained peak plasma values at or above 25 mg/l which was considered satisfactory. Therefore, with the exception of 26 patients, where peak absorption times occurred later than 2.5 h after intake, all pharmacokinetic observations were as expected.

4. Discussion

The current study shows an improvement in tumor control when compared to the similar patient group in the DAHANCA 2 trial [17,34]. Although this may not solely be due to the use of nimorazole, but also be a consequence

Table 5

Loco-regional control (5-year actuarial value) as a function of nimorazole and stratification group or other factors of significance for the radiation response

Stratification group	Placebo		Nimorazole		Stratified *P*-value
	No. of patients	Control (%)	No. of patients	Control (%)	
Sex					
Female	48	45 ± 7	62	56 ± 6	0.003
Male	147	30 ± 4	157	46 ± 4	
Region					
Supraglottic	57	35 ± 6	68	53 ± 7	0.002
Pharynx	138	33 ± 4	151	47 ± 4	
T-classification					
T1–T2	92	42 ± 5	105	54 ± 5	0.001
T3–T4	103	27 ± 5	114	45 ± 5	
N-status					
N0	79	43 ± 6	108	61 ± 5	0.006
N +	116	27 ± 5	111	38 ± 5	
Staging					
Stage 1	5	40 ± 21	16	67 ± 12	0.005
Stage 2	34	51 ± 9	36	59 ± 9	
Stage 3	67	47 ± 7	85	49 ± 6	
Stage 4	89	18 ± 4	82	41 ± 6	
Differentiation					
Well	20	20 ± 9	24	55 ± 10	0.003
Moderate	55	31 ± 6	60	44 ± 7	
Poor/undifferentiated	67	35 ± 6	84	53 ± 6	
Hemoglobin					
High[a]	116	37 ± 5	127	54 ± 5	0.001
Low	79	29 ± 6	92	43 ± 6	
Transfused	34	29 ± 9	48	46 ± 8	0.12
Not transfused	45	30 ± 8	44	40 ± 8	

[a]Females <8, males <9 mmol/l.

of changing the radiotherapy schedule from split-course to continuous irradiation [17,36], it does demonstrate the usefulness of pursuing a constant policy within the nationwide DAHANCA study group.

The beneficial tumor response in favor of nimorazole appears promising. The results are further encouraged by the observation that an apparent independent and additive relationship exists between the use of the hypoxic radiosensitizer and the hemoglobin concentration (Table 5). This is probably due to a combination of a hemoglobin concentration-dependent oxygen delivery to the tumor together with the hypoxic sensitization. This is in agreement with the DAHANCA 2 trial and a striking similarity between the two studies has appeared [34,41]. It is remarkable that the Danish head and neck cancer studies so far have been the only large randomized clinical trials which have shown an apparent benefit of the use of hypoxic radiosensitizers [8,30,32]. There is substantial evidence from hyperbaric oxygen trials, measurements of oxygen concentrations in tumors and the relationship between hemoglobin concentration and tumor control which indicates that hypoxia may be a critical factor when treating carcinomas of the head and neck by radiotherapy [28,30,37]. However, the experience has also pointed towards a substantial het-

Table 6

Cox proportional hazards analysis using loco-regional failure and death from cancer as end-points

	Loco-regional failure		Dead from cancer	
Variable	*P*-value	RR (95% CI)[a]	*P*-value	RR (95% CI)[a]
T1–T2 versus T3–T4	0.0002	1.65 (1.25–2.17)	0.003	1.52 (1.15–2.00)
N0 versus N+	<0.0001	1.84 (1.38–2.45)	<0.0001	2.13 (1.60–2.85)
Placebo versus nimorazole	0.005	0.69 (0.52–0.90)	0.03	0.74 (0.57–0.97)
Males versus females	0.06	NE	0.02	1.49 (1.06–2.09)
High versus low hemoglobin	0.13	NE	0.09	NE
Supraglottic versus pharynx	0.88	NE	0.16	NE

RR, relative risk. NE, not estimated.
[a]Indicates the risk of failure or death for the last mentioned variable relative to the first, e.g. risk of failure for T3–T4 tumors relative to T1–T2 tumors.

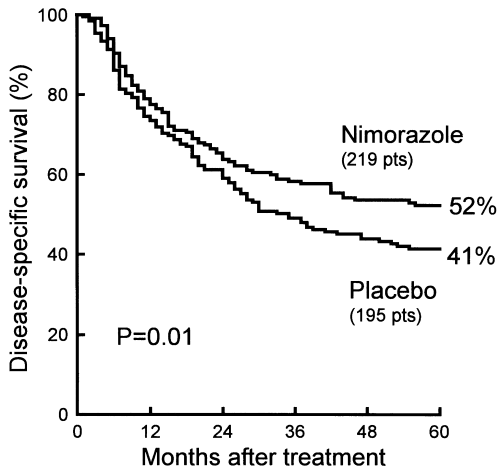

Fig. 5. Actuarial estimated disease-specific survival rate in patients randomized to receive nimorazole or placebo in conjunction with conventional radiotherapy for carcinoma of the pharynx and supraglottic larynx.

erogeneity in the extent of hypoxia within tumors of the same size, site and histopathology [28,40] and it is likely that only in large and homogeneous clinical trials can such differences be detected. The current DAHANCA 5 study has utilized the experience from the previous clinical trial in order to further identify patients with a hypoxia problem and in addition, the two Danish head and neck clinical trials are among the largest randomized studies performed with hypoxic radiosensitizers in head and neck carcinoma so far [32]. Furthermore, no selection has been performed within the patient group since all patients with head and neck carcinomas are treated within the few oncological centers in the country.

Our present results have confirmed the observation from the DAHANCA 2 protocol that modification of hypoxic radioresistance with nitroimidazoles improves the loco-regional control in squamous cell carcinomas of supraglottic larynx and pharynx. The magnitude of this sensitization is significant, taking into consideration the lack of major drug-related toxicity. As a consequence of these two randomized trials nitroimidazole has now become part of the baseline radiotherapy protocol which is used by all institutions in Denmark.

Over the last 30 years a substantial amount of clinical trials have evaluated the hypoxic modification of radiotherapy in head and neck cancer [8,30,32,37]. These include trials using hyperbaric oxygen [18,19] and various nitroimidazoles [10–12,25–27]. Although not consistent, the overall outcome is that such hypoxic modification will result in a significant benefit in loco-regional control and a meta-analysis of all randomized studies has shown an odds ratio of 1.3 [33,37]. The results from the current study also contributed to this meta-analysis, but do not significantly influence its conclusion, which is maintained after elimination of the study from the analysis. As a consequence of the improvement in loco-regional control, the overview also demonstrated a significant improvement in (disease-specific) survival [37]. Thus, substantial evidence exists indicating that hypoxic modification is likely to improve the outcome of radiotherapy in head and neck cancer. Provided such modification can be performed without major morbidity or other difficulties, it will therefore be considered a part of standard therapy as we consider the evidence for its beneficial efficacy to be sufficient.

In contrast to the current results, two more recent large randomized trials evaluating the hypoxic sensitizer etanidazole in head and neck cancer have not demonstrated a significant difference [11,25] and obviously the outcome from various clinical trials are not demonstrating a uniform benefit of hypoxic modification. The reason for this discrepancy should probably be seen in both the heterogeneity of the included tumors and in the efficacy of the hypoxic modification.

The use of nitroimidazoles has major limitations since the most potent drugs are associated with substantial toxicity and therefore can only be given in small and infrequent doses. As previously discussed [32], there is a discrepancy between the preclinical evaluation of new sensitizers and the clinical applicability. In preclinical animal studies the 2-nitroimidazoles have been shown to be the most potent drugs [38]. However, when applied in clinical practice these drugs can only be given in low doses, which in association with a smaller tumor/plasma ratio results in a significant reduction of the drug in the tumor when given in a fractionated treatment. The 5-nitroimidazoles are less active at high concentrations, but in clinically usable doses they seem to yield at least the same extent of hypoxic radiosensitization as more potent drugs. Furthermore, they can be delivered in substantially higher tumor concentrations [32]. The difference between the etanidazole trials and the current study therefore could simply be a consequence of the extent of hypoxic radiosensitization. As previously demonstrated, the amount of drug available in a tumor per fraction in a 30-fraction regime with nimorazole is almost twice that of etanidazole [32].

No important or chronic toxicity has been noted with the use of nimorazole and the drug response and the side-effect profile are in agreement with the previous phase I and II studies [35,39,47].

The compliance to nimorazole in the present trial was less than anticipated, but cannot be explained by drug-related side-effects alone. In addition, there is a substantial placebo effect (Table 7) and it was also observed that 16% of the patients were unable to comply with the drug, mainly due to problems with swallowing the capsules. Both the placebo effect and the large capsules may be related to the double-blind design of the trial because the patients were very carefully instructed about potential side-effects and the capsules had to be made larger in order to make the drug tasteless and blinded. Since the completion of the DAHANCA 5 protocol, nimorazole has been part of the standard treatment of most head and neck cancer patients and their compliance has substantially improved partly due to the use of coated

Table 7
Drug-related toxicity and compliance

Toxicity/compliance	Nimorazole (n = 219) (%)	Placebo (n = 195) (%)	All (n = 414) (%)
Completed as scheduled without any side-effects	107 (49)	140 (72)	247 (60)
Toxicity	77 (35)	24 (12)	101 (24)
Nausea/vomiting	46 **26**	16 **7**	62
Skin rash	12 **8**	3 **1**	15
Flushing	14 **12**	4 **2**	18
Other	5 **4**	1 **1**	6
Other non-compliance (not drug-related)	36 (16)	31 (16)	67 (16)

Numbers in bold typeface indicate patients in whom the treatment was not completed (i.e. <25 drug treatments); nimorazole 83 patients (38%); placebo 40 patients (21%).

pills rather than capsules. The routine use of the drug may therefore reduce the side-effects and increase the compliance.

The evaluation in the present study was based on the 'intention to treat' principle. However, a number of patients in the nimorazole group did not receive the planned amount of drug. A total of 34 patients in the nimorazole group received less than five drug treatments due to either toxicity or refusal (but not due to causes related to their cancer disease or radiotherapy treatment). The response in this patient group was of the same magnitude as the placebo group (5-year loco-regional control rate of 34%), whereas the 5-year loco-regional control rate of the 185 nimorazole-treated patients who actually received the drug was 52%. Thus, an apparent dose response relationship for nimorazole seems to exist.

The compliance to radiotherapy was good and the same in both treatment arms. Unfortunately, the scoring of radiation-related morbidity was rather crude, but no significant difference in either early or late morbidity was observed. However, a more detailed recording of radiation-related morbidity may be useful and has therefore been adapted in the more recent DAHANCA studies.

It has been well established that hypoxia is present in head and neck cancers and that an increased amount of hypoxia is associated with poor outcome after radiotherapy [3,28]. Although such heterogeneity in the hypoxic status is evident there is currently no proper way to predict the presence of hypoxia and/or the ability of individual tumors to respond to hypoxic modification. Until such methods have been developed, the tumors in question must therefore all be considered to be at risk of being hypoxic and candidates for hypoxic modification. Since further development of the hypoxic modification is likely to demand more aggressive treatment, such as the use of carbogen and nicotinamide [7,22,24,43], or more potent hypoxic sensitizers and bioreductive drugs [4,16,23], which unfortunately also have higher toxicity, there is a demand for better methods to identify the relevant hypoxic tumors. Continuous research into the prediction of relevant hypoxia is therefore strongly needed, preferably by the use of techniques which are based on routine diagnostic procedures such as imaging and histopathology [6,46].

The relationship between hemoglobin and tumor response is intriguing, although not uniformly observed in all trials [13]. It is obvious that hemoglobin by itself may not reflect the true oxygen status of the tumor, partly due to variations in tumor blood flow and partly because the oxygen unloading capacity of the blood may widely differ [14,20,29]. The latter may especially be a consequence of smoking habits [14,37]. Since almost all patients with head and neck carcinomas are smokers (also during radiotherapy), variations in oxygen availability may be as substantial in patients with the same hemoglobin value. An analysis of this problem is in progress. However, it should not be forgotten that a low hemoglobin value by itself may just indicate a poorer general condition of the patient, which in turn may lead to a worse prognosis.

The protocol also addresses the issue of the transfusion of low hemoglobin patients to a high hemoglobin level. Whether this will be a successful procedure remains to be analyzed. This will require a more detailed study of the hemoglobin level in transfused patients and this was not performed in the current analysis.

As previously observed [40], this trial also demonstrated that women had a better prognosis. This may be partly explained by a different distribution in stage and tumor site, but an additional independent sex-related prognostic parameter seems to exist.

The potential presence of hypoxia in head and neck tumors may not be the only cause of radioresistance. Proliferation of tumor cells during treatment and intrinsic resistance are also known to be responsible for failure to respond to radiotherapy and reducing the overall treatment time may improve the outcome [1,9,21,31,42]. The difference between the outcome in the DAHANCA 2 and 5 trials should therefore be seen in the light of a poorer tumor control after split-course therapy with an overall treatment time of 9.5 weeks. This effect may especially be found in the well-differentiated tumors [17], whereas the effect of nimorazole appeared to be present irrespective of the histopathological grade (Table 4). Therefore, future strategies towards improving the effect of radiotherapy in head and neck cancer must not only attempt to minimize the influence of hypoxia, but also aim to reduce the overall treatment time. The latter is preferable without reducing the total

Table 8
'Bottom-line' calculation of the effect of nimorazole

	Loco-regional control	Disease-specific survival	Overall survival
Relative risk	0.74	0.73	0.93
Relative risk reduction (%)	26	27	7
Absolute risk reduction (%)	17	16	5
No. of patients needed[a]	6	6	19

[a]Number of patients (on average) needed to treat to achieve the benefit of nimorazole in one patient.

dose to avoid failures due to intrinsic radioresistance. Such a treatment strategy is the basis of the DAHANCA 7 protocol in which patients receiving nimorazole are randomized between radiotherapy given with five or six fractions of 2 Gy per week to the same total dose [42].

The outcome of a randomized trial may be difficult to interpret since the data can be presented in various ways. It may therefore be useful to indicate the outcome in the form of a 'bottom-line' using various effect parameters to illustrate the magnitude of the results [15]. Table 8 shows the calculation of such a 'bottom-line' effect of the use of nimorazole in terms of the likely benefit for the individual patients, taking into consideration that the treatment intervention is economically affordable, has no major side-effects and is easy to administer. Since the intervention is rather simple and can be performed without major problems, it is our conclusion that it has such a magnitude and benefit that it should be part of routine radiotherapy of the relevant tumors until other alternatives have been proven to be more effective.

Acknowledgements

This study was supported by grants from the Danish Cancer Society, Legatstiftelsen Pedersholm and the Danish Cancer Society, Clinical Research Unit at Aarhus Oncological Center.

References

[1] Ang, K.K. Accelerated fractionation: what is the price for speeding? Radiother. Oncol. 44: 97–99, 1997.
[2] Bentzen, S.M. Towards evidence based radiation oncology: improving the design, analysis, and reporting of clinical outcome studies in radiotherapy. Radiother. Oncol. 46: 5–18, 1998.
[3] Brizel, D., Sibley, G.S., Prosnitz, L.R., Scher, R.L. and Dewhirst, M.W. Tumor hypoxia adversely affects the prognosis of carcinoma of the head and neck. Int. J. Radiat. Oncol. Biol. Phys. 38: 285–289, 1997.
[4] Brown, J.M. and Siim, B.G. Hypoxia-specific cytotoxins in cancer therapy. Semin. Radiat. Oncol. 6: 22–36, 1996.
[5] Bush, R.S., Jenkin, R.D.T., Allt, W.E.C., Beale, F.A., Bean, H., Dembo, A.J. and Pringle, J.F. Definite evidence for hypoxic cells influencing cure in cancer therapy. Br. J. Cancer (Suppl. III): 302–306, 1978.
[6] Chapman, J.D., Engelhardt, E.L., Stobbe, C.C., Schneider, R.F. and Hanks, G.E. Measuring hypoxia and predicting tumor radioresistance with nuclear medicine assays. Radiother. Oncol. 1998, in press.
[7] Denekamp, J. and Fowler, J.F. ARCON – current status: summary of a workshop on preclinical and clinical studies. Acta Oncol. 36: 517–525, 1997.
[8] Dische, S. Chemical sensitizers for hypoxic cells: a decade of experience in clinical radiotherapy. Radiother. Oncol. 3: 97–115, 1985.
[9] Dische, S., Saunders, M., Barrett, A., Harvey, A., Gibson, D. and Parmar, M. A randomized multicentre trial of CHART versus conventional radiotherapy in head and neck cancer. Radiother. Oncol. 44: 123–136, 1997.
[10] EORTC Cooperative Group of Radiotherapy. Early results of the EORTC randomized clinical trial on multiple fractions per day (MFD) and misonidazole in advanced head and neck cancer. Int. J. Radiat. Oncol. Biol. Phys. 12: 587–591, 1986.
[11] Eschwege, F., Sancho-Garnier, H., Chassagne, D., Brisgand, D., Guerra, M., Malaise, E.P., Bey, P., Busutti, L., Cionini, L., N'Guyen, T., Romanini, A., Chauvaudra, J. and Hill, C. Results of a European randomized trial of etanidazole combined with radiotherapy in head and neck carcinomas. Int. J. Radiat. Oncol. Biol. Phys. 39: 275–281, 1997.
[12] Fazekas, J., Pajak, T.F., Wasserman, T., Marcial, V., Davis, L., Kramer, S., Rotman, M. and Stetz, J. Failure of misonidazole-sensitized radiotherapy to impact upon outcome among stage III–IV squamous cancers of the head and neck. Int. J. Radiat. Oncol. Biol. Phys. 13: 1155–1160, 1987.
[13] Fazekas, J.T., Scott, C., Marcial, V., Davis, L.W., Wasserman, T. and Cooper, J.S. The role of hemoglobin concentration in the outcome of misonidazole-sensitized radiotherapy of head and neck cancers: based on RTOG trial #79-15. Int. J. Radiat. Oncol. Biol. Phys. 17: 1177–1181, 1989.
[14] Grau, C. and Overgaard, J. Significance of hemoglobin concentration for treatment outcome. In: Medical Radiology: Blood Perfusion and Microenvironment of Human Tumours, pp. 101–112. Editors: M. Molls and P. Vaupel. Springer-Verlag, Heidelberg, 1997.
[15] Greenhalgh, T. Statistics for the non-statistician. II. 'Significant' relations and their pitfalls. Br. Med. J. 315: 422–425, 1997.
[16] Haffty, B.G., Son, Y.H., Sasaki, C.T., Papac, R., Fischer, D., Rockwell, S., Sartorelli, A. and Fisher, J.J. Mitomycin C as an adjunct to postoperative radiation therapy in squamous cell carcinoma of the head and neck: results from two randomized clinical trials. Int. J. Radiat. Oncol. Biol. Phys. 27: 241–250, 1993.
[17] Hansen, O., Overgaard, J., Hansen, H.S., Overgaard, M., Høyer, M., Jørgensen, K.E., Bastholt, L. and Berthelsen, A. Importance of overall treatment time for the outcome of radiotherapy of advanced head and neck carcinoma: dependency on tumor differentiation. Radiother. Oncol. 43: 47–51, 1997.
[18] Henk, J.M., Kunkler, P.B. and Smith, C.W. Radiotherapy and hyperbaric oxygen in head and neck cancer. Final report of first controlled clinical trial. Lancet, ii: 101–103, 1977.
[19] Henk, J.M. and Smith, C.W. Radiotherapy and hyperbaric oxygen in head and neck cancer. Interim report of second clinical trial. Lancet, ii: 104–105, 1977.
[20] Hirst, D.G. Anemia: a problem or an opportunity in radiotherapy? Int. J. Radiat. Oncol. Biol. Phys. 12: 2009–2017, 1986.
[21] Horiot, J.C., Bontemps, P., van den Bogaert, W., Le Fur, R., van den Weijngaert, D., Bolla, M., Bernier, J., Lusinchi, A., Stuschke, M., Lopez-Torrecilla, J., Begg, A.C., Pierart, M. and Colette, L. Accelerated fractionation (AF) compared to conventional fractionation (CF) improves loco-regional control in the radiotherapy of advanced head and neck cancers: results of the EORTC 22851 randomized trial. Radiother. Oncol. 44: 111–121, 1997.
[22] Horsman, M.R. Nicotinamide and other benzamide analogs as agents

for overcoming hypoxic cell radiation resistance in tumours. Acta Oncol. 34: 571–587, 1995.
[23] Horwich, A., Holliday, S.B., Deacon, J.M. and Peckham, M.J. A toxicity and pharmakokinetic study in man of the hypoxic-cell radiosensitiser RSU-1069. Br. J. Radiol. 59: 1238–1240, 1986.
[24] Kaanders, J.H.A.M., Stratford, M.R.L., Liefers, J., Dennis, M.F., van der Kogel, A.J., van Daal, W.A.J. and Rojas, A. Administration of nicotinamide during a five-to-seven-week course of radiotherapy: pharmacokinetics, tolerance, and compliance. Radiother. Oncol. 43: 67–73, 1997.
[25] Lee, D.-J., Cosmatos, D., Marcial, V.A., Fu, K., Rotman, M., Cooper, J.S., Ortiz, H.G., Beitler, J.J., Abrams, R.A., Curran, W.J., Coleman, C.N. and Wasserman, T.H. Results of an RTOG phase III trial (RTOG 85-27) comparing radiotherapy plus etanidazole (SR-2508) with radiotherapy alone for locally advanced head and neck carcinomas. Int. J. Radiat. Oncol. Biol. Phys. 32: 567–576, 1995.
[26] Lee, D.-J., Pajak, T.F., Stetz, J., Order, S.E., Weissberg, J.B. and Fischer, J.J. A phase III study of the hypoxic cell sensitizer misonidazole as an adjunct to high fractional dose radiotherapy in patients with unresectable squamous cell carcinoma of the head and neck: a RTOG randomized study. Int. J. Radiat. Oncol. Biol. Phys. 16: 465–470, 1989.
[27] MRC Working Party on Misonidazole in Head and Neck Cancer. A study of the effect of misonidazole in conjunction with radiotherapy for the treatment of head and neck cancer. Br. J. Radiol. 57: 585–595, 1984.
[28] Nordsmark, M., Overgaard, M. and Overgaard, J. Pretreatment oxygenation predicts radiation response in advanced squamous cell carcinoma of head and neck. Radiother. Oncol. 41: 31–39, 1996.
[29] Overgaard, J. The influence of hemoglobin concentration on the response to radiotherapy. Scand. J. Clin. Lab. Invest. 48 (Suppl. 189): 49–53, 1988.
[30] Overgaard, J. Sensitization of hypoxic tumour cells – clinical experience. Int. J. Radiat. Biol. 56: 801–811, 1989.
[31] Overgaard, J. Advances in clinical applications of radiobiology: phase III studies of radiosensitizers and novel fractionation schedules. In: Head and Neck Cancer, Vol. III, pp. 863–869. Editors: J.T. Johnson and M.S. Didolkar. Elsevier, Amsterdam, 1993.
[32] Overgaard, J. Clinical evaluation of nitroimidazoles as modifiers of hypoxia in solid tumors. Oncol. Res. 6: 509–518, 1994.
[33] Overgaard, J. Modification of hypoxia – from Gottwald Schwarz to nicotinamide. Have we learned the lesson? In: Progress in Radio-Oncology V, pp. 469–475. Editor: H.D. Kogelnik. Monduzzi Editore, Bologna, 1995.
[34] Overgaard, J., Hansen, H.S., Andersen, A.P., Hjelm-Hansen, M., Jørgensen, K., Sandberg, E., Berthelsen, A., Hammer, R. and Pedersen, M. Misonidazole combined with split-course radiotherapy in the treatment of invasive carcinoma of larynx and pharynx: report from the Dahanca 2 study. Int. J. Radiat. Oncol. Biol. Phys. 16: 1065–1068, 1989.
[35] Overgaard, J., Hansen, H.S., Lindeløv, B., Overgaard, M., Jørgensen, K., Rasmusson, B. and Berthelsen, A. Nimorazole as a hypoxic radiosensitizer in the treatment of supraglottic larynx and pharynx carcinoma. First report from the Danish Head and Neck Cancer Study (DAHANCA) protocol 5-85. Radiother. Oncol. 20 (Suppl. 1): 143–149, 1991.
[36] Overgaard, J., Hjelm-Hansen, M., Johansen, L.V. and Andersen, A.P. Comparison of conventional and split-course radiotherapy as primary treatment in carcinoma of the larynx. Acta Oncol. 27: 147–152, 1988.
[37] Overgaard, J. and Horsman, M.R. Modification of hypoxia-induced radioresistance in tumors by the use of oxygen and sensitizers. Semin. Radiat. Oncol. 6: 10–21, 1996.
[38] Overgaard, J., Overgaard, M., Nielsen, O.S., Pedersen, A.K. and Timothy, A.R. A comparative investigation of nimorazole and misonidazole as hypoxic radiosensitizers in a C3H mammary carcinoma in vivo. Br. J. Cancer 46: 904–911, 1982.
[39] Overgaard, J., Overgaard, M. and Timothy, A.R. Studies of the pharmacokinetic properties of nimorazole. Br. J. Cancer 48: 27–34, 1983.
[40] Overgaard, J., Sand Hansen, H., Jørgensen, K. and Hjelm-Hansen, M. Primary radiotherapy of larynx and pharynx carcinoma. An analysis of factors influencing local control and survival. Int. J. Radiat. Oncol. Biol. Phys. 12: 515–521, 1986.
[41] Overgaard, J., Sand Hansen, H., Overgaard, M., Jørgensen, K., Bastholt, L., Berthelsen, A. and Pedersen, M. The Danish Head and Neck Cancer Study Group (DAHANCA) randomized trials with hypoxic radiosensitizers in carcinoma of the larynx and pharynx. In: Radiation Research. A Twentieth-Century Perspective, Vol. II, Congress Proceedings, pp. 573–577. Editors: W.C. Dewey, M. Edington, R.J.M. Fry, E.J. Hall and G.F. Whitmore. ICRR, Toronto, 1991.
[42] Overgaard, J., Sand Hansen, H., Sapru, W., Overgaard, M., Grau, C., Jørgensen, K., Bastholt, L., Hansen, O., Specht, L., Berthelsen, A. and Pedersen, M. Conventional radiotherapy as the primary treatment of squamous cell carcinoma of the head and neck. A randomized multicenter study of 5 versus 6 fractions per week – preliminary report from the DAHANCA 6 and 7 trial. Radiother. Oncol. 40: S31, 1996.
[43] Saunders, M.I., Hoskin, P.J., Pigott, K., Powell, M.E.B., Goodchild, K., Dische, S., Denekamp, J., Stratford, M.R.L., Dennis, M.F. and Rojas, A. Accelerated radiotherapy, carbogen and nicotinamide (ARCON) in locally advanced head and neck cancer: a feasibility study. Radiother. Oncol. 45: 159–166, 1997.
[44] Sealy, R., Jacobs, P., Wood, L., Levin, W., Barry, L., Boniaszczuk, J. and Blekkenhorst, G. The treatment of tumours by the induction of anemia and irradiation in hyperbaric oxygen. Cancer 64: 646–652, 1989.
[45] Stell, P.M. and Rawson, N.S.B. Adjuvant chemotherapy in head and neck cancer. Br. J. Cancer 61: 779–787, 1990.
[46] Thrall, D.E., Rosner, G.L., Azuma, C., McEntee, M.C. and Raleigh, J.A. Hypoxia marker labeling in tumour biopsies: quantification of labeling variation and criteria for biopsy sectioning. Radiother. Oncol. 44: 171–176, 1997.
[47] Timothy, A.R., Overgaard, J. and Overgaard, M. A phase I clinical study of nimorazole as a hypoxic radiosensitizer. Int. J. Radiat. Oncol. Biol. Phys. 10: 1765–1768, 1984.