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Concomitant Vinorelbine and Radiation in Head and Neck Squamous Cell Carcinoma in Vitro

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Concomitant chemoradiotherapy has been used for locally advanced head and neck squamous cell carcinoma (HNSCC) particularly with cisplatin, 5-FU, methotrexate, bleomycin and taxanes. Vinorelbine is a semisynthetic vinca alcaloid, which causes a block in the G2/M phase of the cell cycle. HNSCC cell lines have previously been reported to be sensitive to vinorelbine in nanomolar concentrations. In the current study the effect of vinorelbine as a radiosensitizer in vitro was studied and eight recently established head and neck SCC cell lines of the UT-SCC-series were tested. Vinorelbine concentrations of 0.4–1.6 nM were used, corresponding to the IC70, IC50 and IC30 values of each cell line, resulting in 30%, 50% and 70% inhibition in clonogenic survival. The desired concentrations of vinorelbine were added to the medium and the cells were plated in 96-well culture plates in this solution. The plated cells were irradiated 24 h later with 4MeV photons generated by a linear accelerator and incubated at 37°C with 5% CO_2 for 4 weeks. Thereafter, the number of wells containing coherent, living colonies, consisting of 32 cells or more, was counted. The plating efficiency was calculated and the fraction survival data were fitted to the linear quadratic model [$F = \exp[-(\alpha D + \beta D^2)]$]. An additive effect of combining vinorelbine and irradiation could be demonstrated. The dose-dependent decrease in survival was seen at vinorelbine doses of 0.4–1.6 nM in all cell lines tested.

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Vinorelbine is a unique semisynthetic vinca alcaloid that differs from the naturally occurring compounds, vinblastine and vincristine, in its chemical structure, selectivity for mitotic microtubules and toxicity profile (1). Vinorelbine is a classic anti-tubulin in that its mechanism of action involves arresting mitosis at metaphase by binding to tubulin, leading to the inhibition of tubulin assembly and microtubule formation (2). Thus, it is a cell-cycle-dependent antimitotic agent blocking progression in the G2/M cell phase, which is the most sensitive phase of the cell cycle to irradiation. Clinical studies showed relatively few side effects and neutropenia as the dose-limiting toxicity of vinorelbine. Since vinorelbine has relatively low affinity for axonal microtubules compared to other mitotic inhibitors, its neurotoxicity is mild (3). Vinorelbine has shown a broad spectrum of activity against breast cancer, lung cancer, ovarian cancer and lymphoma (4–6). Currently, vinorelbine is in routine clinical use against breast and lung cancer. In vitro studies showed that vinorelbine is able to potentiate the antitumor effects of radiation in non-small cell lung cancer (7). Furthermore, clinical studies have proved that vinorelbine is a promising radiosensitizer in locally advanced non-small cell lung cancer (8).

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common malignant disease world-wide. The long-term prognosis of patients with advanced head and neck cancer has been poor, not only because of metastatic disease, but also primarily because of failure in locoregional disease control. Traditional therapy for these patients has been surgery and radiotherapy. The use of concurrent chemotherapy and radiation, or chemoradiation, has been clinically investigated since the 1960s. The simultaneous administration of chemotherapy and radiotherapy is theoretically aimed at improving both systemic and locoregional tumor control (9). Most of the studies throughout the 1970s and 1980s have been focused on the use of single-agent chemotherapy during a standard course of single daily fraction radiotherapy (9–11). The single agents most frequently used were methotrexate, bleomycin, mitomycin C, fluorouracil and cisplatin. The use of multiagent chemoradiotherapy has also been studied in patients with advanced HNSCC (12). In fact, various drug combinations and doses as well as radiotherapy treatment schedules have been studied over the past few years (13, 14).

We have shown earlier that head and neck SCC cells are constantly sensitive to vinorelbine in vitro (15). In this

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study, we investigated vinorelbine as a radiosensitizer in head and neck squamous cell carcinoma cell lines.

MATERIAL AND METHODS

Cell culture

Eight head and neck SCC cell lines established by us were tested in this study. The characteristics of the cell lines are presented in Table 1 (16). Before the start of the experiments the cells were maintained in logarithmic growth in T25 culture flasks by passing weekly in Dulbecco's modified Eagle's minimal essential medium (DMEM) containing 2 mM L-glutamine, 1% non-essential aminoacids, 100 Uml^{-1} streptomycin, 100 Uml^{-1} penicillin and 10% fetal bovine serum (FBS). The cells were kept in logarithmic growth by passing them weekly or biweekly.

Drug preparation

Vinorelbine (Navelbine®, provided by Pierre Fabre Pharma Norden AB) 10 mg/ml was diluted with growth medium to give a stock solution of 1.0 μM. Final vinorelbine dilutions of 0.4 nM–1.6 nM were used, and new stock solutions were made for each experiment. In this study we used three different vinorelbine dilutions, which correspond to the IC70, IC50 and IC30 values of each cell line, i.e. the drug concentration causing 30%, 50% and 70% inhibition in clonogenic survival. These IC values were obtained from results of clonogenic assays after fitting the data to the linear quadratic equation, as previously reported (15).

Clonogenic assay and irradiation

The cells were grown in T25 culture flasks into mid-logarithmic phase (40%–60% confluency) and fed with fresh medium on the day before plating for the experiments. The clonogenic assay was performed as described elsewhere (17). In brief, the cells were harvested with trypsin/EDTA, counted and diluted to a stock solution of 4 167 cells/ml. The number of cells plated per well was adjusted according to the plating efficiency (PE) of each cell line. Further dilutions of this single cell suspension either with or without vinorelbine were made in 50 ml of Ham's F-12 medium containing 15% FBS and the desired concentrations of vinorelbine were added to these solutions. The cells in this solution were plated in 96-well culture plates by applying 200 μl/well using an octa-pipette. After plating, the cells were allowed to attach for 24 h before irradiation. To test the concomitant use of vinorelbine and radiation, the cells were treated with vinorelbine for 24 h before irradiation and the drug was allowed to remain in the plates during the whole incubation period.

The cells were irradiated in 96-well culture plates with 4 MeV photons generated by a linear accelerator (Clinac 4/100; Varian, CA); delivering a dose-rate of 2.0 Gy/min (18). In each study with one cell line, four different sets of plates with four repeats were used. The first set was used as a control with no vinorelbine, whereas in the other three sets vinorelbine was added in concentrations corresponding to the IC70-, IC50- and IC30- values of the respective cell lines. Each set consisted of two control plates and two plates with the following radiation doses: 0.75 Gy, 1.25 Gy, 2.5 Gy, 5.0 Gy and 7.5 Gy, respectively. Thus, each set included 12 plates and the whole study with one cell line included 48 plates. Each study with one cell line was repeated at least three times. Detailed dosimetry has been published elsewhere (18). The plates were incubated in a water vaporsaturated atmosphere containing 5% CO_2 at 37°C. After 4 weeks, the number of positive wells was counted using an inverted phase-contrast microscope. Wells with colonies consisting of at least 32 cells were considered positive.

Data analysis

The PE was calculated using the formula ln (number of negative wells/total number of wells)/number of cells plated per well. Fraction survival data as a function of the radiation dose with or without the indicated vinorelbine dose were found to fit to the linear quadratic equation. A microcomputer program was used to fit data to

Table 1

Characteristics of the cell lines

Cell line	Gender	Primary tumor location	TNM*	Specimen site	Type of lesion	Grade	AUC (Gy)
UT-SCC-1A	Female	Gingiva of mandible	T2N1M0	Gingiva of mandible	Recurrence	G2	1.7±0.3
UT-SCC-2	Male	Floor of mouth	T4N1M0	Floor of mouth	Primary	G2	1.8±0.2
UT-SCC-9	Male	Glottic larynx	T2N1M0	Neck	Metastasis	G1	1.4±0.1
UT-SCC-11	Male	Glottic larynx	T1N0M0	Larynx	Recurrence	G2	2.0±0.2
UT-SCC-19A	Male	Glottic larynx	T4N0M0	Larynx	Primary	G2	1.7±0.1
UT-SCC-29	Male	Glottic larynx	T2N0M0	Larynx	Primary	G1	1.8±0.2
UT-SCC-33	Female	Gingiva of mandible	T2N0M0	Gingiva of mandible	Primary	G2	2.3±0.2
UT-SCC-34	Male	Supraglottic larynx	T4N0M0	Supraglottic larynx	Primary	G1	2.1±0.1

*TNM classification according to the International Union against Cancer (UICC, 1977).
Abbreviation: SCC = Squamous cell carcinoma.
Grade 1 (G1) = well differentiated; Grade 2 (G2) = moderately differentiated; Grade 3 (G3) = poorly differentiated.

$F = \exp[-(\alpha D + \beta D^2)]$. The area under the curve (AUC) value, equivalent to mean inactivation dose (D), was obtained by numerical integration. The AUC ratio (AUC for vinorelbine+radiation/AUC for radiation) and the surviving fraction after the indicated dose of vinorelbine were used to compare the effect of combined vinorelbine and irradiation with the effects of vinorelbine alone.

The type of interaction was described by the terms 'additive', corresponding to the effect being commensurate with the calculated effects of the drug and radiation alone ($1+1=2$), and 'supra-additive', when the effect of concurrent use of drug and radiation is considered to be more cytotoxic than the calculated effects of the drug and radiation alone ($1+1>2$). A 'subadditive effect' would indicate less than the expected sum of the individual effect of the drug and radiation ($1+1<2$).

RESULTS

The surviving fractions at IC70, IC50 and IC30 doses of vinorelbine for the 8 cell lines tested and their intrinsic radiosensitivity are listed in Table 2. When concomitantly used with 0.4–1.6 nM vinorelbine, an additive effect with radiation was seen in all cell lines tested. The surviving fraction of vinorelbine alone corresponds to the radiosensitizing effect in concomitant use measured as the correlation of the area under the survival curve of vinorelbine+radiation to that of radiation alone (Fig. 1a–h). The survival curves comparing radiation alone and concomitant vinorelbine and radiation are clearly parallel in all cell lines tested. Thus, an additive effect but not supra-additivity was noticed, since the effect of simultaneous vinorelbine and radiation was of the same magnitude as that calculated by combining the cytotoxic effects of the two modalities alone. The observed, modest differences in chemosensitivity between the cell lines did not affect the vinorelbine-radiation synergy. Nor was the type of interaction modulated by the intrinsic radiosensitivity of the cell lines (Table 1) or the increasing dose of vinorelbine

DISCUSSION

The poor prognosis for advanced head and neck carcinoma indicates the need for the development of multimodality therapies including surgery, radiotherapy and chemotherapy. For this task, knowledge of the radiobiologic characteristics of head and neck carcinoma and its sensitivity to different chemotherapeutic agents is essential. In vitro techniques such as clonogenic assays provide the best means for elucidating these properties in preclinical studies. In the present investigation, we evaluated the radiosensitizing effect of vinorelbine on 8 head and neck SCC lines in vitro.

Table 2

Concomitant vinorelbine and radiation. The drug concentrations used represent the IC70, IC50 and IC30 values for each cell line (15). Survival fraction for each cell line and for each vinorelbine concentration used as well as the additive effect of vinorelbine

Cell line	Vinorelbine dose (nM)	Surviving fraction (S(O))±SD	AUC vinorelbine+radiation ±SD AUC radiation
UT-SCC-1A	0.5 (IC70)	0.72±0.14	0.66±0.12
	0.7 (IC50)	0.51±0.17	0.40±0.10
	1.1 (IC30)	0.33±0.16	0.26±0.08
UT-SCC-2	0.5 (IC70)	0.83±0.12	0.74±0.10
	0.7 (IC50)	0.55±0.15	0.55±0.08
	0.9 (IC30)	0.38±0.07	0.42±0.07
UT-SCC-9	0.5 (IC70)	0.73±0.21	0.61±0.10
	0.9 (IC50)	0.47±0.09	0.41±0.08
	1.4 (IC30)	0.37±0.12	0.31±0.06
UT-SCC-11	0.5 (IC70)	0.78±0.12	0.84±0.10
	0.7 (IC50)	0.66±0.06	0.67±0.08
	1.1 (IC30)	0.47±0.08	0.38±0.08
UT-SCC-19A	0.5 (IC70)	0.84±0.12	0.88±0.06
	0.9 (IC50)	0.52±0.05	0.50±0.07
	1.4 (IC30)	0.39±0.10	0.35±0.09
UT-SCC-29	0.4 (IC70)	0.92±0.08	0.88±0.06
	0.6 (IC50)	0.59±0.09	0.66±0.08
	1.0 (IC30)	0.41±0.06	0.39±0.08
UT-SCC-33	0.5 (IC70)	1.0±0.05	1.0±0.06
	1.0 (IC50)	0.84±0.08	0.76±0.05
	1.5 (IC30)	0.59±0.12	0.53±0.08
UT-SCC-34	0.5 (IC70)	0.90±0.06	0.88±0.05
	1.0 (IC50)	0.74±0.07	0.63±0.08
	1.6 (IC70)	0.35±0.15	0.34±0.10

Abbreviation: AUC = area under the curve.

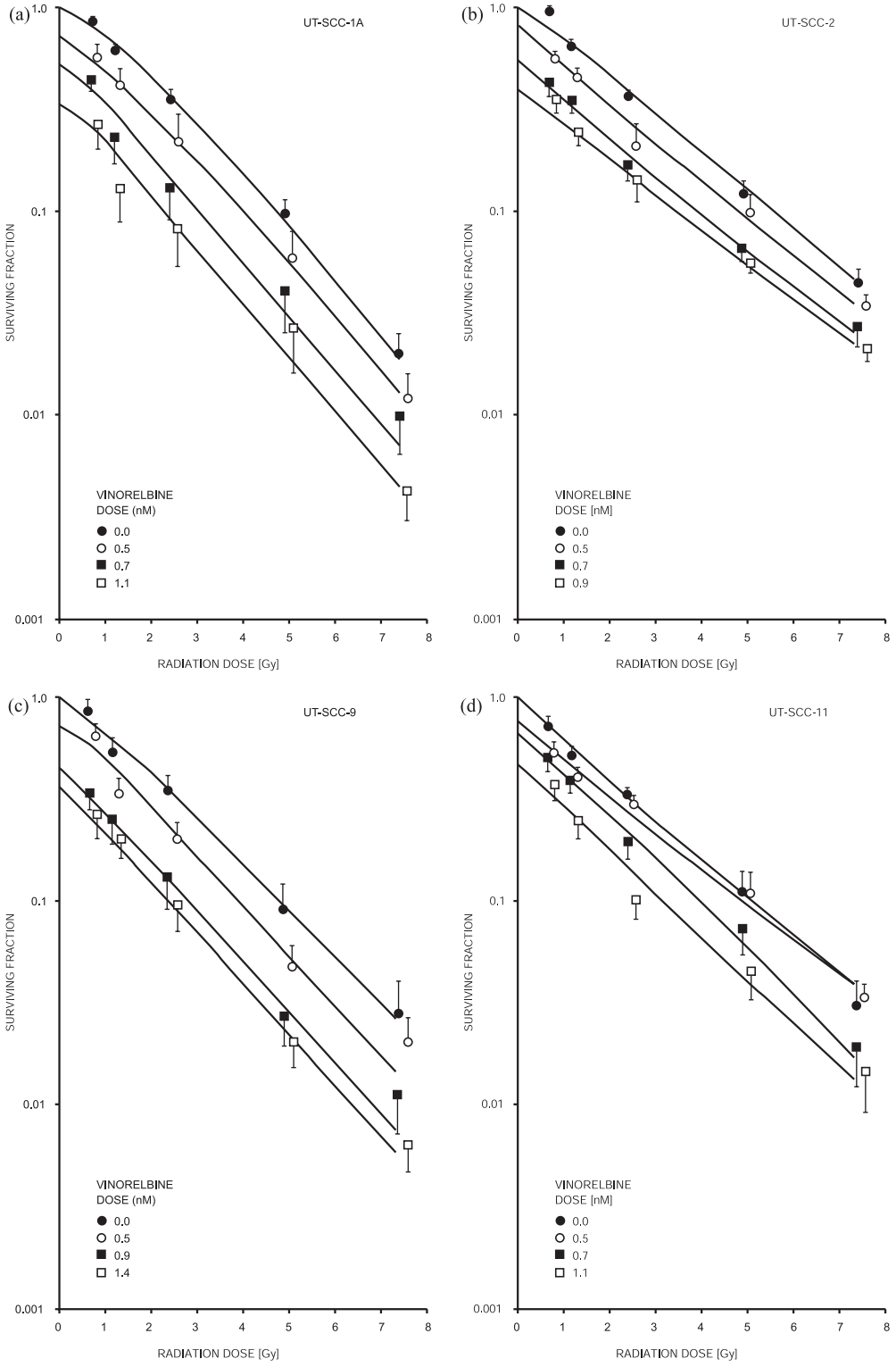

Fig. 1a–h. Radiosensitivity curve and the sensitivity to concomitant vinorelbine and radiation in the 96-well plate clonogenic assay of the 8 squamous cell carcinoma cell lines studied.

Our results showed a strictly additive growth inhibiting effect when the cells were exposed to radiation and vinorelbine, simultaneously.

HNSCC in vitro is constantly sensitive to vinorelbine. The chemosensitivity of these head and neck SCC cell lines to vinorelbine expressed as IC50, corresponding to the drug

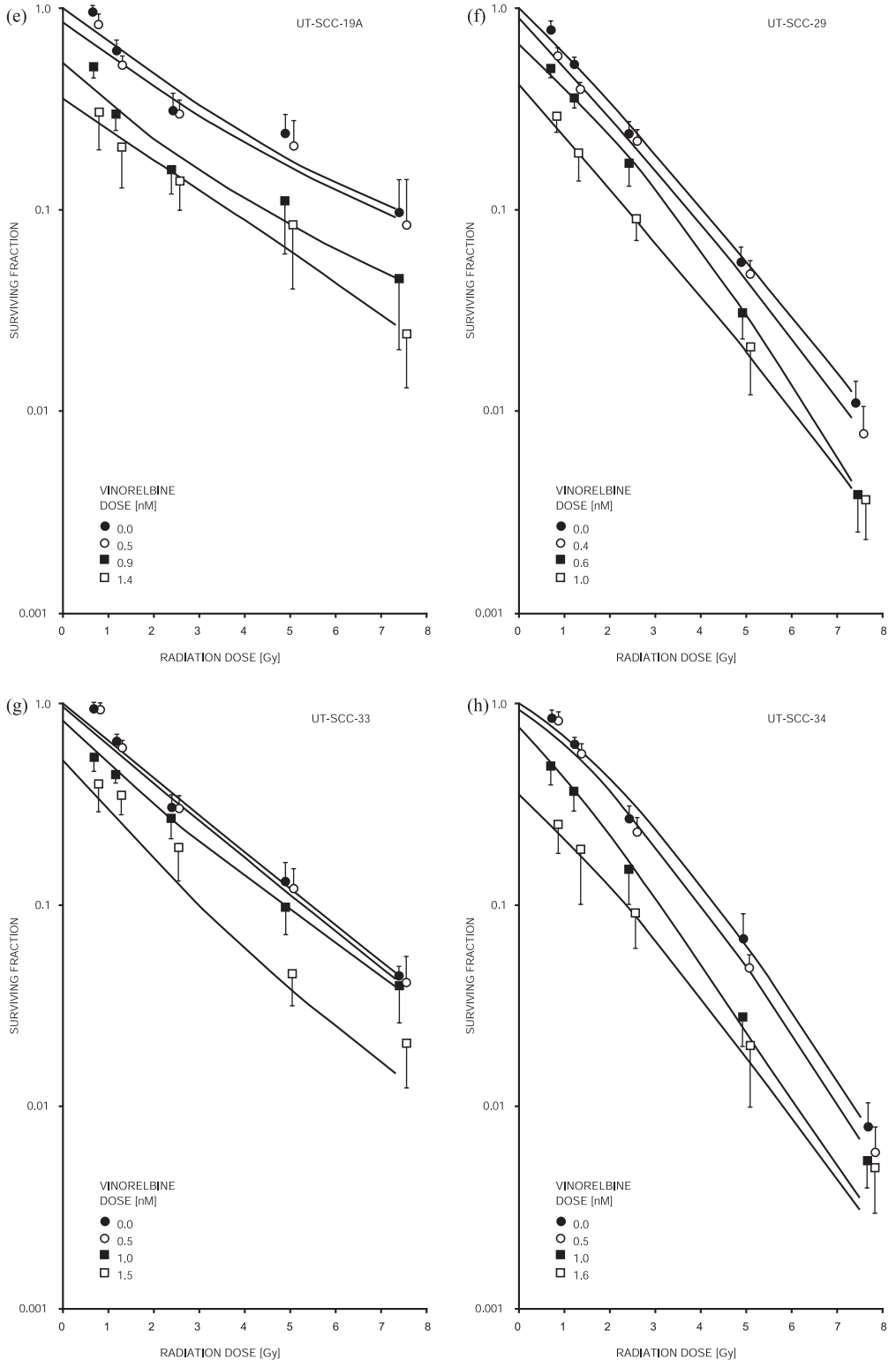

Fig. 1a–h (*Continued*)

concentration causing 50% inhibition in clonogenic survival, varied between 0.6 and 1.0 nM (15). In our previous studies the chemosensitivity to carboplatin varied from 0.65 μM to 2.96 μM in 5 UT-SCC cell lines (19) and the chemosensitivity to paclitaxel varied from 1.2 to 2.9 nM in 8 UT-SCC cell lines studied (20). These studies point out the low variance in sensitivity of HNSCC in vitro to vinorelbine.

Concurrent use of radiation and chemotherapeutic agents for the treatment of head and neck cancer patients has been an area of interest for several investigators over the past few years. In clinical trials, 5-FU, cisplatin and paclitaxel used in combination with radiation has been shown to be a feasible combination giving significantly better locoregional tumor control with improved overall survival in the management of head and neck cancer (12, 14). The results of some trials also indicate that vinorelbine in combination with 5-FU and cisplatin followed by hyperfractionated irradiation is a potential and active drug in locally advanced HNSCC (21). In planning future treatments of patients with head and neck cancer, several goals must be considered, including improved survival, quality of life, and organ function. Concurrent chemotherapy and radiotherapy might also offer an alternative to surgery and reconstruction for patients with resectable tumors in sites where conservation of organ function is desirable.

HNSCC in vitro is constantly sensitive to vinorelbine, which has an additive effect in concomitant use with irradiation. Vinorelbine is an interesting compound for clinical studies of concomitant chemo-irradiation also because it is available in oral form allowing new and also frequent drug administration schedules.

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