CHEMOTHERAPY

IN

CANCER OF THE ESOPHAGUS

THESIS

T.C. KOK

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CHEMOTHERAPY IN CANCER OF THE ESOPHAGUS

CHEMOTHERAPIE BIJ SLOKDARMKANKER

PROEFSCHRIFT

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de Erasmus Universiteit Rotterdam

op gezag van de rector magnificus

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en volgens besluit van het college voor promoties.

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"... en zie, Prinses, de lucht klaart op
wij gaan nu tot de aanval over -
want nu hebben wij gekozen
en de getijden van het lot zijn in beweging ..."

aan mijn ouders

DANK

Ate	voor nimmer aflatende inspanningen
Ted	voor inspirerende gedachten
Kees	voor onbaatzuchtige hulp
Gerrit	voor geduld

DANK

Connie	en collega's voor "info-on-demand"
maten	in de Rotterdams Slokdarm Werkgroep
	voor geestdrift

DANK

320	
mensen	met slokdarmkanker voor hoop en geloof

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anderen	voor brandstof en begrip

CONTENTS

Although cancer of the esophagus has been recognized as a fatal disease as long ago as the start of the Christian era, the present outlook remains dismal. Less than 10 percent of patients with seemingly localized disease, surgically treated with curative intent, will survive five years or more. Dysphagia, the initial symptom in most patients, usually occurs late in the course of the disease, when the esophageal wall has been infiltrated or penetrated. At this time, in the majority of patients, metastases are present in surrounding lymphe nodes and/or other organs.

Until recently, chemotherapy had no role in the treatment of this disease. It was generally used as a last resort, after primary surgery or radiation therapy, in patients with a poor performance status. With the availability of new drugs, used in various combinations, and sometimes in conjunction with other forms of cancer treatment, a modest success is standing out against the horizon.

This thesis includes an overview of the current knowledge on chemotherapy in esophageal cancer (chapter 1), several clinical studies on the efficacy and toxicity of various drugs and combinations of drugs in metastatic esophageal cancer (chapters 2-5), a comparative study on preoperative chemotherapy in patients with operable esophageal cancer (chapter 6), and an exploratory study, to evaluate the benefit of salvage surgery after chemotherapy (chapter 7) in patients with metastatic esophageal cancer. In addition, two laboratory experiments on the presence of human papilloma virus and the role of the Multidrug Resistance Protein in esophageal cancer are covered (chapters 8,9).

CHAPTER I

CHEMOTHERAPY IN ESOPHAGEAL CANCER:

A REVIEW

TC Kok

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The disease.

Squamous cell carcinoma of the esophagus exhibits a most striking variation of incidence among different regions in the world. Prevalence rates between the most- and least- affected areas differ approximately 500-fold (0.4/100.000 for women in Utah (USA) vs 170/100.000 in some regions in Iran). (1) Specific areas of high incidence include Iran (Azerbadzjan) and certain regions of the former USSR (Kazakhstan and Turkmenistan) towards China, forming the so called Central Asian Cancer Belt, France (Normandy and Brittany), Kenya, Zimbabwe and South Africa. (2) Even within a very short distance, high-risk areas adjoin much lower-risk regions, questioning the influence of environmental factors, like population, local agriculture, climate, vegetation, and geology. (3) Until now a variety in risk factors have been found, notably low consumption of fresh fruit and vegetables, consumption of food contaminated by mycotoxins and nitrosamines, and the consumption of alcohol and tobacco. Nitrosamines are potent carcinogens in the laboratory animal. (4) Apart from their low levels in food, these compounds can be formed in vivo by an interaction of amines and nitrites. Nitrites have been used in preservation of meats, but can also be formed from nitrate by bacterial reduction during storage of food. (5) The effects of the consumption of alcohol *per se*, although generally believed to be a major cause of esophageal cancer in Europe and in the USA, are far from clear. Spirits have been found to present a much greater hazard than that resulting from consumption of the same amount of ethanol in the form of wine or beer. (6) Another complicating factor is the synergistic effect of tobacco smoking on alcohol related esophageal cancer, as simultaneous exposure to alcohol and tobacco is a common occurrence. Smoking as a independent risk factor has been regarded plausible,(7) as well as not convincing. (8)

Last but not least, it is well established that alcohol consumption can lead to nutrient defiency potentially increasing the vulnerability of the esophagus to carcinogens. Results from a case-control study of blacks in Washington, DC, with a high incidence of esophageal cancer (29/100.000), showed that the least well-nourished individuals had twice the risk of developing esophageal cancer as the most well-nourished. (9) On the other hand, a good diet is not always totally protective against esophageal cancer, as can be seen in Calvados, France, where an exceptionally high incidence (45/100.000) of esophageal cancer is observed in an affluent population with a high consumption of spirits. (10)

Micronutrient deficiencies possibly associated with (esophageal) cancer are zinc, riboflavin, and some vitamins, but a deficiency of one of these compounds *per se* does not appear to be a sufficient cause of esophageal cancer. Zinc deficiency has been associated with an increase in the mitotic index of the esophageal and buccal mucosa whereas, surprisingly, atrophy and a decrease in rate of cell proliferation can occur in other organs, including the small intestine. (11) Riboflavin deficiency can result in reduced enzyme levels (like flavine adenine dinucleotide) which play a crucial part in the activity of many oxidases and dehydrogenases, essential for the integrity of epithelial cells. These enzymes are also involved in the mixed-function oxidase system, responsible for the detoxification or activation of many carcinogens.

Little is known about the exact role of viruses, especially papilloma viruses (HPV). In 1985 a possible etiologic relation between HPV and proliferations of the squamous mucous membrane of the esophagus was suggested. (12)(13) Since then, a number of controversial studies have been published about the detection of HPV DNA in human esophageal cancer with different techniques. (14)(15)

An important observation is the changing pattern in type of cancer during the last two decades. The age-adjusted incidence

rates for squamous cell carcinomas are decreasing, while a very rapid increase in the incidence rate of distal adenocarcinomas can be seen especially in white men in the Western World. (16)(17) The reason for this emergence is not well understood. Frequently, a history of hiatal hernia or chronic gastrointestinal reflux is reported by patients, but the significance of this is unclear. (18) Barrett's esophagus, a condition in which squamous epithelium is replaced by an abnormal columnar epithelium, however, has been shown to predispose to the development of esophageal adenocarcinoma. (19)(20)

Several reviews, discussing the role of predisposing factors in esophageal cancer have been published, (21)(22)(23); a remarkable attempt to indicate a structure in the jig-saw of possible factors in the etiology of esophageal cancer has been performed recently by Craddock.(24)

Diagnosis and staging.

Dysphagia is the predominant symptom in more than 80% of patients. Other symptoms are regurgitation or vomiting and discomfort in the throat, substernal area or epigastrium in about half the patients. Thirty percent of the cases suffer from retrosternal pain, often produced or worsened by the alimentary transit. Hoarseness points to a recurrent laryngeal nerve involved by cancer. Esophageal cancer can be diagnosed by means of upper gastrointestinal endoscopy and biopsies, sometimes after barium contrast X-ray examination. Most tumors can be classified as either squamous cell carcinoma or adenocarcinoma. Small cell carcinomas and adenoid cystic carcinomas are uncommon variants. (25) Grading of differentiation has not yet been recognized as an important prognostic factor.(26) Staging is based on the UICC TNM staging system,

which in fact describes the anatomical extent of the disease .(27) The T-category points to the depth of infiltration of the primary tumor through the esophageal wall, which can be determined very accurately with endoscopic ultrasonography (EUS).(28) If EUS is hampered by a narrow stricture in the esophagus, bronchoscopy should be performed, especially when the tumor is located in the middle third of the esophagus, to rule out tracheo-bronchial invasion.(29) The N-category is related to the presence or absence of regional lymph node involvement. A physical examination, with special attention to the supraclavicular lymph nodes (which are defined as regional in the case of a cervical esophageal tumor), should be followed by an ultrasound examination of this region and the upper abdomen, with ultrasound-guided biopsies if necessary. (30) A CT-scan of the chest and upper abdomen has been proven accurate, especially in detecting distant metastases (M-category).(31)(32)

Local treatment modalities: surgery and radiotherapy.

At present, *surgery*, by means of an esophagectomy followed by a reconstruction, is the treatment of choice for patients with carcinoma confined to the esophagus. Although being a challenge for both the surgeon and the patient, operative mortality rates are nowadays in specialized centers below 10 %. Esophagectomy can be performed by a transthoracic or by a transhiatal procedure, the latter being recommended for those patients for whom thoracotomy would be hazardous.(33) This blunt resection has a potential disadvantage; a complete lymph node dissection cannot be garanteed, with a risk of understaging the patient. A reconstruction conduit can be fashioned, usually with the stomach. In patients with previous gastrectomy, the colon is the most appropriate replacement. Survival after surgery is stage-dependent: 65-85% for stage I-II (UICC) to

10-25% for stage III patients.(27)(34) Several reviews exist on the surgical treatment of esophageal cancer.(35)(36)

External beam *radiation therapy* (XRT) as a single treatment modality remains only modestly effective. Results of XRT and surgical series cannot be compared because of patient- and tumor-selection. Median survival is 6-12 months with <10% 5-year survival. Local control at the primary tumor site fails in 50-75% of cases. (37) Although endo-esophageal intraluminal brachytherapy can be used as a boost to external beam XRT, survival after this combined radiation program in one series was only improved in selected patient populations. (38)

Preoperative radiotherapy, given in an attempt to increase the chance of resectability and reduce the risk of pre-operative dissemination of tumor cells, was first reported in 1960. (39)

Several reports showed an increase in the resectability rate from ± 50 percent to ± 75 percent after comparison with historical controls. (40)(41)(42)(43) In subsequent randomized trials a somewhat higher resectability rate and reduction in locoregional failure after resection was observed, but there was no indication of even a minimal impact on survival (44)(45)(46).

The experience with *postoperative radiotherapy* has been of limited benefit in prolonging survival.(47)(48) At present, adjuvant radiation therapy in general is not warranted; a proportion of patients with microscopic tumor left behind after surgery (R1 resection) could benefit from such a treatment. (49)

Systemic treatment: chemotherapy.

Many patients with seemingly localised esophageal cancer will have recurrences or metastatic disease despite aggressive local treatment given with curative intent. It is generally accepted that in the Western population, where $T_{3-4}N_{+}$ tumors are observed frequently, 50-70% of the patients have systemic disease at presentation. Autopsy reports show lymph node metastases to be the most common type of spread seen in 40-70%, followed by more distant metastases in the liver and lungs.(50)(51)(52) The use of systemic treatment, alone or in combination with local modalities thus seems rational. Because of the relative rarity of this disease in the West, the severe symptoms in many patients at the time of diagnosis, and the rapidity of tumor progression, esophageal cancer has not been systematically tested against a variety of cytostatic drugs. Nevertheless promising data appear in the literature, especially on combination chemotherapy in multimodality programs. Two thorny issues appear and re-appear amongst the reports: possible chemosensitivity differences between squamous cell- and adenocarcinomas, and the problem of assessment of response in the absence of a measurable mass. With regard to the first, historically, the results of most single agent trials have been restricted to squamous cell cancers. In current reports and meta-analyses, evaluating recently developed drugs or drug combinations, both overall response rate and response rate by histological subtype are increasingly reported.(53)(54)(55)(56)(57) Randomized multimodality trials, such as the recently closed US Intergroup trial 0113 and the ongoing UK MRC trial (preoperative chemotherapy in both squamous cell cancer and adenocarcinoma) should give more insight into possible differences in chemosensitivity.

The second issue concerns the criteria for response evaluation, particularly in patients without measurable disease.

This applies to patients with tumors confined to the esophagus or peri-esophageal region for example. Relief of dysphagia, as used in older reports, has now been abandoned as a major response criterion, although it will remain important, especially as a contributing factor to quality of life. (58) Radiographic evaluations using barium esophagrams have been advocated,(59)(60) right up to mathematical precision,(61) but incorporating other diagnostic tools like endoscopy with biopsies, endoscopic ultrasound, and computerized tomography (CT-scan) seems useful, since these techniques amplify each other, at least partially.(62). In the mean time, technical limitations have also been revealed, for instance with endoscopic ultrasound, with wich overstaging the T-stage after response to chemotherapy and radiotherapy has been reported .(63)(64)(65) Only formal reponse evaluations in prospective studies will provide data of sufficient reliability to reach a consensus about the requirements for a valid "measurement" of an unmeasurable parameter.(66)

Several extensive reviews have been published about the results achieved so far with chemotherapy, especially after single agent treatment with classical cytostatics in metastatic patients.(67)(68)(69) Although the response evaluations should be comparatively reliable in this category, in which measurability disease is a prerequisite, a number of uncertainties have not been elucidated. In many early reports the number of treated patients is small; response rates usually decrease in larger study populations. With some agents, no clear data exist on the dose-response relationship, as may be the case for instance with methotrexate.(70)(71) Last but not least, calculating and reporting the duration of response was not considered necessary at that time.

Table 1 summarises the available data on the most active single agents in disease oriented studies with reasonable numbers of patients.

Table 1. Chemotherapy in esophageal cancer / single agents.
(S=squamous cell carcinoma A = adenocarcinoma)

drug	reference	number of patients	response (%)	S / A
Bleomycin	(60) (72) (73) (74)	10+14+29+15=64	2+0+4+4=10 (15%)	S/S/S/S
Cisplatin	(75) (76) (77) (78) (79)	17+24+35+26+45 =147	1+6+9+4+5=25 (17%)	S/S/S/S/S
Etoposide	(80) (81)	6+26=32	0+5=5 (15%)	S/S
5-Fluoro-uracil	(70) (82) (83)	23+13+29=65	4+12+5=21 (32%)	S/S+A/A
Methyl-GAG	(84) (85) (86)	23+20+21=64	4+5+6=15 (23%)	S/S
Mitomycin-C	(76) (87)	24	10 (41%)	S/S+A
Methotrexate	(70) (71)	26+41=64	3+20=23 (34%)	S/S
Paclitaxel	(55)	50	16 (32%)	A+S
Vindesine	(88) (89) (90) (91)	23+9+51+46=129	4+1+14+6=25 (19%)	S/S/S?/S
Vinorelbine	(92)	46	7 (15%)	S

Bleomycin has several potentially dangerous side effects. Pulmonary toxicity, especially when thoracic surgery or radiotherapy is anticipated, prohibits its wide use in combined modality programs. Although **Cisplatin** seems to form the axis round which a number of combinations is currently being developed, the total response rate was in several studies less than 20%. In one trial, 22 of 40 patients (55%), who were treated before operation, showed tumor necrosis in the resection specimens.(93) Neurotoxicity can be a limiting factor, especially in patients with a history of longstanding alcohol abuse. The drug has a comparatively favourable toxicity profile, with bone marrow toxicity primarily affecting red blood cells. All the single agents listed in table 1 have been studied in combination with cisplatin, resulting in response rates of 35-50% (table 2). In addition cisplatin has been studied as a radiation sensitizer, often in combination with **5-fluoro-uracil**. Although first developed in 1958, this agent is still under study regarding its optimal dose and schedule. In recent years several mechanisms have been elucidated, by which its activity can be enhanced through addition of *biochemical modulators*, such as leucovorin and α-interferons. **Methyl-GAG** or mitoguazone exerts a potent effect on the bone-marrow, thereby putting itself out of combined modality approaches. **Mitomycin-C** has similar disadvantages, with risks of prolonged thrombocytopenia and a potentially lethal hemolytic-uremic-syndrome. However, dose limitations largely avoids these, and with high single agent activity the drug can be justified for use in combinations regimens. Two studies describe the activity of **Methotrexate**, and suggest a dose-response relationship; this would need to be confirmed in a randomised study before any recommendations regarding high dose therapy could be made. **Paclitaxel** has proven active in a well conducted phase II trial with promising results in both adeno- and squamous cell tumors. This drug is now being studied in combinations with cisplatin and 5-fluoro-uracil. **Vindesine** has

shown a consistent but moderate activity as single agent, with neuropathy as a common side effect. This is less frequent with **Vinorelbine**, a new semisynthetic vinca alkaloid, with 26% neurotoxicity grade 1 (WHO) in the first phase II study. Studies combining this agent with cisplatin, are underway. Agents with little or no documented activity in esophageal cancer include **Carboplatin** (94)(95)(96)(97), **Iproplatin** (98)(99), and the **Anthracyclines** (100)(101).

Confronted with the moderate response rates observed with single agent chemotherapy in esophageal cancer so far, many feel that the way forward is with combination regimens, accepting that the real merits of a given agent in both types of histology have not been fully elucidated. Many combinations have already been reviewed (67)(68)(69) and are summarised in table 2.

Table 2. Chemotherapy in esophageal cancer / combination regimens.
(S=squamous cell carcinoma A = adenocarcinoma)

drug	reference	number of patients	response (%)	S/A
cisplatin + ara-C	(102)	16	6 (38%)	S
cisplatin + ara-C + 5-fluorouracil	(103)	32	13 (41%)	A
cisplatin + bleomycin	(104) (105) (106)	61+29+47=137	9+15+9=33 (24%)	S/S/S
cisplatin + bleomycin + methotrexate	(107) (108)	10+31=41	5+8=13 (31%)	S/S
cisplatin + bleomycin + methotrexate + mitoguazone	(109)	14	9 (64%)	S
cisplatin + bleomycin + vindesine	(110) (111) (112) (113)	68+27+17+38=140	36+7+8+21 =72 (51%)	S/S/S/S
cisplatin + bleomycin + 5-fluorouracil	(114)	38	23 (60%)	S
cisplatin + vindesine	(115)	31	5 (16%)	S

cisplatin + vindesine + mitoguazone	(116)	39	16 (41%)	S
cisplatin + vinblastine + mitoguazone	(117) (118)	36+34=70	4+16=20 (28%)	S/S
cisplatin + methotrexate	(71) (119) (120)	42+88+17=147	32+66+14= 112 (76%)	S/S/S
cisplatin + methotrexate + vincristine	(121)	28	19 (67%)	S
cisplatin + etoposide (+/- floxuridine)	(122) (123)	16+65	10+31 (50%)	?/S
cisplatin + etoposide + doxorubicin	(124)	26	13 (50%)	A
cisplatin + mitomycin-C + ifosfamide	(125)	43	19 (44%)	S
cisplatin + 5-fluorouracil	(126) (127) (128) (79) (129) (130) (131)	37+35+39+35+73+- 32+60=311	13+13+14+16+42 +21+19=138 (44%)	S/S/S/ S/S/S/S
cisplatin + 5-fluorouracil + (epi)adriamycin	(132) (133)	21+115=136	7+73=80 (58%)	S/A

cisplatin + 5-fluorouracil + etoposide (+/- fol.acid)	(134) (66) (122) (135)	20+34+15+38=107	13+17+10+ 17=57 (53%)	S/A/?/ S+A
cisplatin + interferon α-2A + 5-fluorouracil (+/- fol.acid)	(56) (136) (137)	27+11+23=61	13+3+15= 31 (50%)	S+A/S+ A/S
cisplatin + paclitaxel + 5-fluorouracil	(138)	9	9 (100%)	S+A
5-fluorouracil + interferon-α 2A	(53) (139)	37+20=57	10+5=15 (26%)	S+A/S+A
interferon-α 2A + 13-cis-retinoic acid	(140) (141)	9+10= 19	0+0=0 (0%)	S/S

From the results summarised in this table it is reasonable to conclude that esophageal cancer is a chemosensitive tumor. Cisplatin + 5-fluorouracil has been studied widely, and in some reviews this treatment has been advocated as the standard for squamous cell cancer. Other cisplatin based regimens have shown at least equal efficacy, for instance cisplatin + methotrexate; the dissimination of experiences with this combination has however been limited to one center, with the possible risk of selection bias. As in the studies with single agents, responses after combination chemotherapy have seldom been subdivided by histology. Nevertheless, the impression exists that adenocarcinoma is somewhat less responsive than squamous cell cancer. The preliminary data on combinations with newer drugs, such as paclitaxel, specify the response by subtype, and appear more promising in patients with adenocarcinoma. In conclusion, combination chemotherapy can result in response rates between 35% and 55%. No randomized trials have been published in which chemotherapy has been compared with best supportive care alone. The median duration of response in most trials with combination chemotherapy is less than 6 months, from which one could infer that chemotherapy should be offered only within the context of a clinical trial. Quality of life assesment should be an essential part of these studies.

Neo-adjuvant chemotherapy.

What is the impact of timing of systemic therapy on outcome? Should conventional post-operative treatment be employed, as in node-positive breast cancer, or is there a rationale for pre-operative or *neo-adjuvant* systemic treatment? Post-operative combination chemotherapy after primary local treatment for esophageal cancer has not been successful yet, although, until now, regimens have been used which are not the most

active. After an esophagectomy however, whether by thoracotomy or by a transhiatal approach, many patients cannot be treated with systemic chemotherapy for a considerable time. Therefore, it is difficult to comply with the basic rule of adjuvant chemotherapy, that is to start systemic treatment as quickly as possible. The concept of neo-adjuvant chemotherapy has been developed to induce early tumour regression, with improved local control when followed by subsequent surgery and/or radiotherapy, and an ability to identify responding and non-responding patients.(142)(143) In addition, several animal-studies in a variety of tumour types have shown an increase in the labelling index of metastases after resection of the primary, and a better survival when chemotherapy was given before resection.(144)(145)(146)(147) Lastly, chemotherapy is likely to have a greater impact when given early in the course of the disease, when subclinical metastatic burden is low and the patient is best able to tolerate toxic side-effects. Also patients die of metastases more often than local relapse alone. (50)(51)(52)

Potential drawbacks of neo-adjuvant chemotherapy include: the (theoretical) possibility of delay in achieving local control; a risk of tumour spread from the primary site in case of a chemoresistant tumour; and the creation of an "unnatural" tumour area with necrosis and fibrosis at the time of surgery in cases of chemosensitive cancers. Although neo-adjuvant systemic treatment appears to be safe, and operability, resectability and post-operative mortality are comparable with surgery alone, no clear survival benefit could be demonstrated in several phase II trials. It must be emphasized however, that this type of study, by its nature, is not appropriate for testing survival differences.(127)(130)(148)(149) Several prospective randomized trials, with small numbers of patients, have been published, comparing neo-adjuvant chemotherapy followed by surgery versus surgery alone.(112)(150)(151) A significant survival benefit from neo-adjuvant treatment

was not detected, although predictably responding patients had prolonged survival, when compared with non-responders. A large scale trial from Hong Kong, with 2 cycles of neo-adjuvant chemotherapy versus surgery alone, gave similar results.(152)
An interim analysis of a similar study, in which the duration of pre-operative chemotherapy was dependent on the response evaluation after 2 cycles of chemotherapy with cisplatin and etoposide, was published recently in abstract form.(153) With a median follow-up of 17.5 months there was a statistically significant benefit in median survival after chemotherapy, not only for responding patients, but also for the chemotherapy group as a whole. Final results with a longer follow-up are awaited. Two large scale phase III trials are now underway. the US Intergroup trial (Nr 0113) randomizing more than 400 patients with squamous cell carcinoma and adenocarcinoma to receive 3 neo-adjuvant and 2 post-operative chemotherapy cycles with cisplatin and 5-fluoro-uracil versus surgery alone, has recently stopped patient entry; results will be available in 1997. An ongoing European trial (MRC, UK) is investigating the effect of 2 cycles of chemotherapy with cisplatin and 5-fluorouracil followed by surgery; no definite results from this trial will be available in the short-term.

Chemo-radiation.

During the last 15 years, a large number of publications have been dedicated to the combination of chemotherapy and radiotherapy. Terminology and definitions have not always been clear. If one tries to achieve an improved therapeutic strategy with chemo-radiation, this can be done in a variety of ways. For instance, one can treat local disease effectively with radiotherapy, while using chemotherapy for distant disease outside the irradiation field, assuming the two treatments do not interact negatively in terms of toxicity. In this

way, the number of treatment schedules combining chemotherapy and radiotherapy is large. If one combines chemotherapy and radiotherapy, aiming at an enhancement of tumor response, the situation can exist, that a drug "sensitises" tumor cells for radiotherapy, without being effective itself. (154) Another, and more complicated stituation, is when both chemotherapy and radiotherapy produce a particular effect on their own, and the effect of the combination appears to be greater than would be expected, for instance by shrinking of the tumor by drugs, enabling smaller radiation field-sizes and higher radiation doses, or inhibition of repair of radiation damage in tumor cells.(155) Most of the time, these processes can be studied-with *concurrent* use of chemo- and radiotherapy, further mentioned in this review as *chemo-radiation*. The drugs, which have been used in chemo-radiation protocols, are most of the time 5-fluorouracil , cisplatin, and mitomycin-C. Compared with chemotherapy studies in patients with metastatic disease, the results of chemo-radiation are more difficult to interpret because of the often retrospective character of the studies, different study groups (locally advanced or metastatic), the absence of clinical response evaluations after the combined treatment, or the addition of prior (neoadjuvant) or subsequent chemotherapy, surgery and/or radiotherapy (table 3).

Table 3. Chemo-radiation.

reference	number of patients/-stage S=squamous A=adeno	chemotherapy	radiotherapy	local response C=-Clinical P=Pathological S=Symptomatic	median survival (-months) res=resection XRT=radiother chemo=chemoth
Byfield (156)	6/III,IV (S)	5-FU 20 mg/m²/d x 5, week 1,3,5-,7,9,11	60 Gy (200 cGy/d x 5, week 1,3,5,7,9,11)	CR 5/6 (C)	not stated
Earle (157)	40/I-III (S)	bleomycin 15 mg/d before XRT, total 210 mg	60 Gy (200 cGy/d x 5, week 1-6)	14/40 (S)	6
Kolaric (158)	15/III,IV (S)	doxorubicin 30 mg/m² day 2,3 week 1,3,6 bleomycin 15 mg/m² day 1,2 week 1,3,6	40 Gy (200 cGy/d x 5, week 1-4)	CR 3/15 (C) PR 6/15 (C)	not stated
Franklin (159)	30/I,II (S)	5-FU 1000 mg/m²/d x 5, week 1,5 mitomycin-C 10 mg/m² day 1	30 Gy (200 cGy/d x 5, week 1-3)	CR 6/18 (P) PR 3/18 (P)	19 (res. +/- XRT)

Leichman (160)	19/I-III (S)	5-FU 1000 mg/m²/d x 5, week 1,5 cisplatin 100 mg/m² day 1,29	30 Gy (200 cGy/d x 5, week 1-3)	CR 5/19 (P)	18 24 (res)
Popp (161)	21/I-IV (S)	5-FU 1000 mg/m²/d x 5, week 1,5 cisplatin 100 mg/m² day 1,29 OR mitomycin-C 10-15 mg/m² day 1,29	30 Gy (200 cGy/d x 5, week 1-3)	CR 5/21 (C/P) PR 13/21 (C)	8.5 12.5 (res)
Lokich (82)	13/I-III (S)	5-FU 300 mg/m²/d week 1-10	45-60 Gy (200 Gy/d x 5, week 6-10)	CR 2/12 (P) CR 10/12 (C) (PR 11/13 after 5FU alone)	16 22% (3 yrs)
Leichman (162)	20/I-III (S)	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 1,29 mitomycin-C 10 mg/m² day 57 bleomycin 20 U/d x 4, week 9,12	30 Gy (200 cGy/d x 5, week 1-3) Boost: 20 Gy (200 cGy/d x 5, week 15,16	CR 11/20 (C)	22
Poplin (163)	102/I-III (S)	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 75 mg/m² day 1, 29	30 Gy (150 cGy/d x 5, week 1-3)	CR 18/71 (P)	12 14 (res)
Coia (164)	30/I,II S:23 A:7	5-FU 1000 mg/m²/d x 4, week 1,5 mitomycin-C 10 mg/m² day 2	60 Gy (200 cGy/d x 5, week 1-6)	CR 26/30 (C)	22 47% (2 yrs)
Coia (164)	20/III,IV S:10 A:8	5-FU 1000 mg/m²/d x 4, week 1,5 mitomycin-C 10 mg/m² day 2	38-60 Gy (200 cGy/d X 5, week 1-6)	14/17 (S)	8

John (165)	21/III,IV (S)	5-FU 1000 mg/m²/d x 4 week 1,3,5 mitomycin-C 10 mg/m² day 1, 36 cisplatin 75 mg/m² day 22	40 Gy (200 cGy/d x 5, week 1-4)	CR 8/14 (C)	11
Richmond (166)	25/I-IV (S:≥ 20)	5-FU 1000 mg/m²/d x 4, week 1,5,9 cisplatin 100 mg/m² day 1,29,56	55 Gy (200 cGy/d x 5, week 1-3, 8-10)	CR 13/23 (C)	12 37% (2 yrs)
Kolaric (167)	27/III,IV (S)	cisplatin 30 mg/m²/d x 4, week 1,3	40 Gy (200 cGy/d x 5, week 1-4)	CR 4/27 (C) PR 11/27 (C)	10+
Seydel (168)	41/I-III (S)	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 1	30 Gy (200 cGy/d x 5, week 1-3) (+ 20 Gy (200 cGy/d x 10) if vital tumor in resection specimen)	CR 11/27 (P)	13 (+/- res/-XRT) 15% (2-yrs; +/- res/XRT)
McFarlane (169)	22/I-IV S:9 A:13	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 1,29 adenoca: + mitomycin-C 7.5 mg/m² day 1,29	30 Gy (200 cGy/d x 5, week 1-3)	CR 8/22 (P)	66% (2 yrs; (res. +/- XRT)
Coia (170)	8/I,II (A)	5-FU 1000 mg/m²/d x 4, week 1,5 mitomycin 10 mg/m² day 2	60 Gy (200 cGy/d x 5, week 1-6)	CR 7/8 (C)	15 29% (3 yrs)
Coia (170)	9/III,IV (A)	5-FU 1000 mg/m²/d x 4, week 1,5 mitomycin-C 10 mg/m² day 2	40-56 Gy (200 cGy/d x 5, week 1-6)	8/9 (S) PR 2/8 (C)	11
Parker (171)	33/I-IV (S)	5-FU 1000 mg/m²/d x 4, week 1 (+2) mitomycin-C 10 mg day 1 (+8)	30 Gy (200 cGy/d x 5, week 1-3)	CR 11/33 (P)	33% (2 yrs)

John (172)	30/I-III S:26 A:4	5-FU 1000 mg/m² x 4, week 1,4,8 mitomycin-C 10 mg/m² day 1,57 cisplatin 75 mg/m² day 29 5-FU 600 mg/m² day 71,85,99 methotrexate 200 mg/m² day 71,8-5,99 leucovorin 10 mg/m² day 72,86,1-00	41.4 Gy (180 cGy/d x 5, week 1-5, 8)	CR 23/30 (C)	12 29% (2 yrs)
Chan (173)	21/II-IV S	5-FU 1000 20 mg/kg/d x 4, week 1,7 mitomycine-C 8 mg/m² day 1	45 Gy (225 cGy/d x 5, week 1,2,7,8)	CR 18/21 (C)	13 28% (2-yrs)
Van Lackey (174)	15/I-III S	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 2,36	30 Gy (200 cGY/d x 5, week 1,2,3)	CR 8/15 (P)	66% (2 yrs; res)
Stewart (175)	29/I-IV S:25 A:4	5-FU 1000 mg/m²d day 1-4 cisplatin 100 mg/m² day 1 mitomycin-C 10 mg/m² day 1	45 Gy (250 cGy/d x 5, week 1-4)	CR 12/25 (C) CR 5/13 (P)	10 (res)
Seitz (176)	35/I-III S	5-FU 1000 mg/m²/d x 5, week 1,5 cisplatin 70 mg/m² day 2, 30	40 Gy (400 cGY x 5, week 1,5)	CR 25/35 (C)	17 41% (2 yrs)
Bidoli (177)	65/I-IV S	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 1, 29	30 Gy (200 cGy/d x 5, week 1,2,3)	CR+PR 49/65 (C)	42% (2 yrs; res. +/- chemo-XRT)

Araujo (178)	28/II S	5-FU 1000 mg/m²/d day 1-3 mitomycine-C 10 mg/m² day 1 bleomycin 15 U/wk (im) week 1-5	50 Gy (200 cGy/d x 5, week 1-5)	CR 21/28 (C)	64% (1 yr) 38% (2 yrs) 16% (5 yrs)
Coia (179)	57/I,II S:39 A:16	5-FU 1000 mg/m²/day x 4, week 1,5 mitomycin-C 10 mg/m² day 2	60 Gy (200 cGy/d x 5, week 1-6)	CR 40/57 (C)	18 29% (3 yrs) 18% (5 yrs)
Coia (179)	33/III,IV S:19 A:12	5-FU 1000 mg/m²/day x 4, week 1,5 miitomycin-C 10 mg/m² day 2	38-60 Gy (200 cGy/d x 5, week 1-6)	23/30 (S)	8
Herskovic (37)(180)	61/I-III S:51 A:10	5-FU 1000 mg/m²/d x 4, week 1,5-,8,11 cisplatin 75 mg/m² day 1,29,50-,71	50 Gy (200 cGy/d x 5, week 1-5)	35/61 (S)	12 38% (2 yrs)
Gill (181)	36/I-IV S:25 A:9	5-FU 800 mg/m²/d x 5, week 1,4 cisplatin 80 mg/m² day 1,21	54-60 Gy (200 cGy/d X 5, week 1-6)	CR 54% (C) PR 38% (C)	14
Forastiere (182)	43/I-III S:22 A:21	5-FU 300 mg/m²/d x 4, week 1,2,3 cisplatin 20 mg/m² day 1-5, 17-21 vinblastine 1 mg/m² day 1-4, 17-20	37.5 Gy (250 cGy/d x 5, week 1-3) OR 45 Gy (2 x 150 cGy/d x 5, week 1-3)	CR 10/41 (P)	29 35% (5 yrs)

Stewart (183)	23/I-IV (A)	5-FU 800 mg/m²/d x 4, week 1,4 leucovorin 4x50 mg/m²/d x 4, week 1,4 cisplatin 100 mg/m² day 1,29 (+ etoposide 25 mg/m² day 1,29 in 13 pts)	30 Gy (200 cGy/d x 15, week 1,2,3)	CR 6/23 (P)	26 (res) 76% (2 yrs; res)
Naunheim (184)	28/I-III (A)	5-FU 500 mg/m²/d x 5, week 1-4 cisplatin 20 mg/m²/d x 5, week 1,4	36 Gy (180 cGy/d x 5, week 1-4)	CR 8/28 (C) CR 4/24 (P)	18 (res) 28% (3 yrs; res)
Bates (185)	35/I-III S:28 A:7	5-FU 1000 mg/m²/d x 4, week 1,5 cisplatin 100 mg/m² day 1	45 Gy (180 cGy/d x 5, week 1-5	CR 18/35 (P)	26 (res) 41% (3 yrs; res)
Hejna (186)	30/III-IV S:23 A:7	cisplatin 30 mg/m²/d x 4, week 1,5,9,13,17 etoposide 120 mg/m²/d x 4, week 1,5,9,13,17	50 Gy (200 cGy/d x 5, week 5-9)	CR 3/30 (C) PR 9/30 (C)	9
Walsh (187)	52/I-IV (A)	5-FU 15 mg/kg/d x 5, week 1,6 cisplatin 75 mg/m² day 7,48	40 Gy (267 cGy/d x 5, week 1-3)	CR 13/52 (P)	32 (res) 37% (3 yrs; res)

Having a glance at the available data on chemo-radiation, with respect to the above mentioned limitations (table 3), one can see that in selected series, in which a surgical resection has been offered to patients responding to chemo-radiation therapy, a pathological complete remission can be achieved in 30% (median; range 16%-53%). In more than half of the patients a complete remission, clinically evaluated by means of barium esophagram, endoscopy and CT-scan, has been reported (median 57%; range 10%-87%). Dysphagia, the most important and debilitating symptom of esophageal cancer, can be treated effectively with chemo-radiation in 75% of stage III,IV patients (median; range 35%-88%). The duration of this response varies from 4-10 months. Survival ranges from 6-22 months for all patients with a median of 8.5 months. When a resection is performed after chemo-radiation, in selected patients, the median survival rises to 18.5 months (range 10-32 months), and the 2 year survival is still 42% (range 15-76%). The stated three year survival rates after chemo-radiation (cisplatin + 5-fluoro-uracil; 30-46 Gy) followed by surgery are 28,37,41, and 66 months respectively. (184)(187)(185)(169) Histology does not appear to be a important prognostic factor regarding response and survival following chemo-radiation therapy. Uncontrolled studies comparing chemo-radiation with radiotherapy alone, did suggest a benefit from chemo-radiation in terms of local response rate and survival; as yet this benefit has not been proven in some older randomized trials, (157)(178) but was positively confirmed in a more recently published study with a longer follow-up. (37)(180)

The toxicity of chemo-radiation can be substantial, but appears to be dependent on the choice of drugs and their schedule of administration. Acute toxicity, like esophagitis and stomatitis, is in most studies greater than that seen with radiation or chemotherapy alone. Severe hematological toxicity can be anticipated by optimizing the treatment schedule. Late toxicity appears to be comparable to that seen with radiation

alone, and consists primarily of stricture formation, which can be treated with dilatation procedures. (188)

Conclusions and perspectives.

Although at this time surgery remains the standard treatment for operable esophageal cancer, it seems clear that chemotherapy will have a major role in the near future. Both squamous cell cancer and adenocarcinoma of the esophagus appear to be chemosensitive. Cisplatin, 5-fluoro-uracil, and possibly Methotrexate and Mitomycin-C, are the most active single agents at this time. New drugs, like Paclitaxel and Vinorelbine are under investigation; the preliminary results are promising. Higher response rates (35-50%) can be seen with combination chemotherapy, most of the time with cisplatin based regimens. The median duration of response is still short with a maximum of 6 months, thereby excluding chemotherapy as a standard treatment for patients with metastatic esophageal cancer at this time.

Because many patients with esophageal cancer die of metastases more often than local relapse, the concept of neo-adjuvant chemotherapy is now being explored in patients with operable cancers. It appears to be safe, and resectability and postoperative morbidity and mortality after neoadjuvant chemotherappy are comparable with surgery alone. Data from several large phase III trials have to be awaited before any conclusion can be made regarding a possible survival benefit.

Effective combination regimens, with a high complete response rate, should be designed, which can be administered to a group of patients with a moderate performance score in a relatively short period of time. The optimal dose-intensity and schedule of administration of systemic treatment in a multimodality

approach should be further investigated; the moderate number of complete remissions in trials performed to date, could have been caused by relative "undertreatment": too few cycles of not very effective regimens. Although a response to chemotherapy may not be a totally independent prognostic factor, an intensification of the known chemotherapy schedules with bone marrow support by colony stimulating factors with the aim of increasing the response rate, should be issues for forthcoming research.(124) In the meantime the discovery of new tools to differentiate between responding and non-responding patients as early as possible in the period of pre-operative treatment, perhaps even before the start of treatment, should be pursued.

The place of postoperative systemic treatment is still unclear; classical adjuvant treatment with cytostatics after an esophageal resection will be difficult, but maybe a consolidation treatment with less toxic drugs (interferons, retinoids) will be of value in eradicating microscopic residual disease. Only randomized clinical trials incorporating control arms and quality of life measurements, will give an answer to these questions, and hopefully a better outlook for the patient. (57)

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CHAPTER 2

CISPLATIN AND ETOPOSIDE IN ESOPHAGEAL CANCER: A PHASE II STUDY

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SUMMARY

In the search for effective chemotherapy regimens which can be used in multimodality treatment programs for patients with cancer of the esophagus, we conducted a phase II trial to determine the activity and toxicity of the combination of cisplatin and etoposide in patients with advanced squamous cell carcinoma of the esophagus. Seventy three consecutive patients with unresectable or metastatic squamous cell carcinoma of the thoracic esophagus were treated with cisplatin 80 mg/m^2 by 4-hour infusion on day 1, etoposide 100 mg (fixed dose) by 2-hour infusion on day 1 and 2, and etoposide 200 mg/m^2 orally on day 3 and 5. Courses were repeated every 4 weeks, for a maximum of 6. The oral dosages of etoposide were modified individually until a significant degree of myelosuppression was reached. Of 65 evaluable patients, 5 complete responses (CR) and 26 partial responses (PR) were seen, for an overall reponse rate of 48% (95% confidence interval, 35 - 60%). Median time to progression was 7 months (range 3 - 72+ months). There were 2 toxic deaths (neutropenic sepsis). The response rate equals that of other cisplatin based regimens. Its toxicity profile allows addition of a third active drug such as 5-fluorouracil.

INTRODUCTION

Cancer of the esophagus is an uncommon disease in Western countries. In contrast, the disease is among the most frequently occurring malignancies in China, Japan, Asia, and South Africa. The age adjusted mortality (3.4 persons per 100,000) in the USA is nearly similar to the incidence: 3.9 persons per 100,000 (Roth *et al.*, 1993:776). The mortality/incidence ratio in the Netherlands is 1.07 for males and 0.99 for females, with an incidence of approx. 900 in 1990 and a

male/female ratio of 2.5 (Visser *et al.*, 1990:66). Most patients are in their 5th to 7th decade of life. A long-standing history of cigarette abuse and heavy alcohol intake is strongly associated with the development of esophageal cancer, in particular with esophageal squamous cell carcinoma (ESCC). Although approximately half of the patients present with localized disease, many of them will have recurrences or metastatic disease despite agressive local treatment; the 5-year survival rate after radical resection is only 10-15% (Müller *et al.*, 1990). Obviously, there is a need for effective systemic treatment. In reviews on single agent activity with cisplatin, 5-FU, bleomycin and mitomycin, the response rate appears to be 15-20%, with a short duration of response (3 months) (Roth *et al.*, 1993:805). Etoposide showed promising activity in ESCC in a phase I study, although a phase II study with a low-dose schedule in pretreated patients could not confirm these early results (Radice *et al.*, 1979; Coonley *et al.*, 1983). However, with a higher dose in non-pretreated metastatic patients, considerable activity was documented (Harstrick *et al.*, 1992). Based on these data and our previously reported experience that the combination of cisplatin and etoposide is safe and effective in non-small cell lung cancer (Splinter *et al.*, 1986), we have performed a phase II study with the combination of these 2 drugs in patients with advanced and/or metastatic squamous cell carcinoma of the esophagus.

PATIENTS AND METHODS

Patient selection

All patients who entered the study were required to have inoperable or metastatic histologically proven squamous cell or undifferentiated non-small cell cancer of the esophagus. Further eligibility criteria were age ≤ 75 years, performance status WHO 0 - 2 , a life expectancy of more than 3

months, a reasonable food passage , bidimensionally measurable disease (or evaluable disease if the primary tumor was the only indicator lesion), WBC count ≥ 3 x 10^9/L, platelets ≥ 100 x 10^9/L, creatinine clearance ≥ 60 ml/min. Prior chemotherapy was not allowed. Patients with overt brain metastases or an irradiated primary tumor as the sole evaluable lesion were excluded. All patients gave informed consent. The protocol was approved by the Dutch Cancer Society.

Treatment

The intravenous (iv) treatment consisted of prehydration with 1500 ml of saline/glucose (0.45%/2.5%) and 4 grams of magnesiumsulfate over 14 hours, followed by 100 mg (fixed dose) etoposide dissolved in 500 ml saline 0.9% given over 2 hours (day 1). Cisplatin 80 mg/m^2 dissolved in 1000 ml saline 0.9% was then administered over 4 hrs, followed by posthydration with saline/glucose 3500 ml over 24 hours. During this posthydration period another 100 mg (fixed dose) of etoposide dissolved in 500 ml saline 0.9% was given over 2 hours, 24 hours after the first dose of etoposide (day 2). After this iv treatment, patients were discharged. Oral treatment consisted of etoposide (capsules of 50 mg), 200 mg/m^2/day on days 3 and 5, in 3 equal parts on each day (10 AM, 2 PM and 6 PM). In case of stenosis with difficulty in swallowing , the content of the capsules was dissolved in lemonade.

In case the WBC-nadir remained above 2 x 10^9/L and/or the platelet-nadir above 100 x 10^9/l, the oral doses of etoposide were increased until a significant nadir (WBC 1.0 - 2.0 x 10^9/L and/or platelets 25- 100 x 10^9/L) was reached. This was done in order to counterbalance possible differences in bio-availability of oral etoposide. In case of WBC-nadir < 1.0 x 10^9/L and/or platelet-nadir < 25 x 10^9/L a 25% dose reduction of oral etoposide was carried out in the next and subsequent courses.

Courses were postponed one week if WBC < 3.5 x 10^9/L and/or

platelets < 100 x 10^9/L on day one of the next planned course. If after 2 weeks of delay WBC and/or platelets had not recovered, patients went off treatment, but were followed for time to progression and survival. No colony stimulating factors were used in this study.

In case of severe neurotoxicity (WHO grade ≥ 3) or renal insufficiency (WHO ≥ 2), treatment was stopped permanently. Routine anti-emetic support consisted of 10 mg dexamethasone before and after administration of cisplatin in combination with domperidon and lorazepam orally. Sometimes 5-hydroxytryptamine receptor antagonists were administered; these drugs were not yet routinely available in the study period.

Courses were repeated every 4 weeks until progression, or up to a maximum of 6.

Efficacy and toxicity

Response evaluation was done according to standard WHO criteria (WHO, 1979). Complete response required complete disappearance of all known tumor for at least 4 weeks, including negative biopsies taken at endoscopy from previous tumor sites. Partial response required a > 50% reduction of the product of the perpendicular diameters of all measurable lesions, or a regression of more than 50% of the tumor volume if the primary tumor in the esophagus was the only evaluable parameter, for at least 4 weeks (Agha *et al.*, 1986). Stable disease required a < 50% reduction or < 25% increase in the size of indicator lesions. Progressive disease was defined as a > 25% increase in the size of tumor lesions or the appearance of a new lesion. Time to progression and survival were calculated from the first day of treatment. Patients were evaluated for response after 2 courses of chemotherapy, or earlier if treatment was stopped due to severe toxicity. Evaluation of tumor response was done after every 2nd course. If progression of disease was evident after one course, the patient was classified as having early progressive disease.

Toxicity was evaluated according to standard WHO criteria at the day of retreatment (WHO, 1979).

RESULTS

Patients

Between July 1985 and October 1991, 73 patients entered the study. Patient characteristics are listed in table 1. At the time of diagnosis, 60 patients had metastatic disease with the primary tumour in situ, and 3 patients had irresectable primary tumors only. Ten patients had developed distant metastases at a median time of 10.5 months (range 3 - 81 months) after local treatment (esophageal resection (n=3); radiotherapy alone (n=2); radiotherapy followed by esophageal resection (n=5). Two patients were not evaluable for response and toxicity because of treatment refusal and loss to follow-up after the 1st course for other than tumor or treatment related reasons. Six patients were not evaluable for response because of tumor related complications (lethal hematemesis in the presence of normal WBC and platelets after the 1st course (n=1), formation of fistulas between the primary tumor and trachea and pleura respectively after the 1st course with no change of disease (n=2), toxic death (neutropenic sepsis before the first response evaluation (n=2), or WHO grade 3 neurotoxicity after the first course (n=1). Therefore 71 patients were evaluable for toxicity and 65 patients for response.

Response

Table 2 shows the tumor response in 65 evaluable patients. The overall response rate was 48% (95% confidence interval (CI), 35 - 60%), including 5 CRs (8%) and 26 PRs (40%). All patients with a CR had measurable tumor lesions, and 3 of them had a primary tumor in situ which also disappeared. Of

the 26 PRs, 23 patients had measurable metastases; 3 had a primary tumor only which was evaluated by endoscopy. In 23 of 31 responding patients, a > 50% tumor regression was observed after the first 2 cycles.

If one includes the patients in a "intent-to-treat" analysis, two toxic deaths and 1 early death should be considered treatment failures, whereas 1 additional patient achieved a CR, and 2 had SD. In that case, the overall response rate is 32 out of 71 patients (45%; 95% CI, 33 - 57%), including 6 CRs (8%).

The median time to progression in 17 responding patients (13 PR, 4 CR), who did not receive additional treatment after chemotherapy, was 6.9 months (range 3-72+ months). In 11 responding patients (10 PR and 1 CR), who were treated with radiotherapy (n=9: esophagus and supraclavicular regions) or surgery (n=2: transhiatal esophagus resection) after chemotherapy, the median time to progression was 11 months (range 5-18 months). In 3 patients time to progression could not be assessed.

Toxicity

A total number of 252 courses was given to 71 patients, evaluable for toxicity (median 4 courses, range 1-6 courses). Treatment was discontinued after 6 courses, according to the protocol (n=18); in case of no further regression after 2 subsequent cycles of chemotherapy (n=14); and in case of progressive disease (n=20). In one patient chemotherapy was discontinued for other than tumor or treatment related matters.

There were 2 toxic deaths (3%) due to neutropenic sepsis. Three other patients died suddenly during treatment because of hypovolemic shock due to massive upper digestive tract bleeding with normal platelet counts. In none of these 3 patients autopsy was permitted.

Seven patients discontinued treatment without evidence of

progressive disease because of intractable vomiting (n=1), neurotoxicity gr 3 and/or renal toxicity gr 3 (n=3), or deterioration of general condition after 5 courses (n=3). Other reasons for discontinuation were pneumonia, perforation caused by a tube insertion, and esophageal-tracheal or -bronchial fistulas.

Fourteen cycles (5%) had to be postponed (median number of days: 8.5 days); 8 cycles (3%) because of cytopenia, 3 cycles because of a recent infection period, and 3 cycles because of moderate cardiac insufficiency in 2 patients. The oral dose of etoposide could be escalated in 58 cycles, and had to be reduced in 36 cycles.

Table 3 shows hematologic toxicity. Severe (WHO grade 3 and 4) leucopenia and thrombopenia were not encountered after the 2nd course any more because of dose modifications of orally given etoposide in subsequent courses as stated in the protocol. Non-hematologic toxicity data are listed in table 4. 5-HT3 receptor blockers were rarely given throughout the study period which probably explains grade 3 and 4 nausea and vomiting in 38% of the patients, and 20% of the cycles. Alopecia was common. Diarrhea was infrequent. Two periods of grade 4 infection and leukopenia occurred: both patients died of pneumonia due to aspiration. All periods of grade 3 infection were related to the lungs. In half of the periods of grade 2 infection, no focus could be determined; other periods were related to the lungs (n=4) and the urogenital tract (n=2). One patient with a longstanding alcohol abuse experienced severe neuropathy (WHO 3) after the first course. In half of the patients mild to moderate increases in serum creatinine were seen.

Survival

All patients, except one, have died. This patient with a primary tumor in situ and pathologically confirmed metastases in the left cervical region, reached CR after 4 cycles. He is

alive and well, without any evidence of disease after ≥ 72 months.

The median survival time in all patients (n=73) from the start of treatment was 8.5 months (range 0.5-72+ months). Nineteen patients (26%) survived for more than 1 year. The median survival time in responding patients without consolidation treatment (radiotherapy, surgery) was 10 months (range 3.0-72+ months), as compared to a median of 5.5 months (range 1.0-26.4 months) in non-responding patients.

DISCUSSION

Notwithstanding a substantial decrease in postoperative mortality after esophageal resection in the last 15 years, long term survival rates in patients with esophageal cancer are still very low as a consequence of the systemic nature of this disease. As a result of the relative rarity and the poor performance status of most patients, data on systemic treatment in esophageal cancer are scarce. No controlled trials of chemotherapy versus best supportive care have been reported.
Cisplatin as a single agent in ESCC was reported for the first time in 1980 (Davis *et al.*, 1980; Ravry *et al.*, 1985; Engstrom *et al.*, 1983). The SWOG reported an overall response rate of 26% among 35 evaluable patients (6 PR and 3 CR) with a regimen of 50 mg/m^2 cisplatin on day 1 and 8 (Panetierre *et al.*, 1984). The median response duration in these trials was 3-4 months. Despite the limited value of compiled trial data, an overall response rate of 25% with single agent cisplatin in esophageal cancer seems credible (Ajani, 1994a). The dose-limiting toxicity is neurotoxicity (especially in patients with high alcohol intake) and ototoxicity.

Because of encouraging results of etoposide in patients with ESCC in phase I studies, Harstrick et al studied in 26 patients a dose regimen of 200 mg/m^2 iv on 3 consecutive days,

which yielded 5 partial responses with a duration of 3,4,5,5, and 8 months (Harstrick *et al.*, 1992). Half of the patients experienced grade 3 leukopenia as major toxicity. No severe organ toxicities were recorded. Higher fractionations of etoposide could lead to higher activity, as has been shown by several authors (Clark, 1992). Other single agents like bleomycin, 5-fluoro-uracil, mitomycin, methotrexate, and vindesine, are effective in only 15-20% of the cases, with no substantial survival benefit (Roth *et al.*, 1993:805). Recently some new agents have been tested: vinorelbine, carboplatin, iproplatin, and paclitaxel. With vinorelbine in non-pretreated patients with squamous cell carcinoma, 6/24 obtained a partial remission; these results have not yet been confirmed (Conroy *et al.*, 1993). Negative results have been reported with the platinum analogs, carboplatin and iproplatin (Sternberg *et al.*, 1985; Steel *et al.*, 1988; Mannell, 1989; Cappelaere *et al.*, 1993). Preliminary results from a phase II trial with paclitaxel have shown interesting activity: an overall response rate of 31% (95% CI, 17-45%, no CR) with a median response duration of 4 months (range 1-11+ months) was recorded (Ajani *et al.*, 1994b). In the clinic, cisplatin appears to be an excellent drug for combination chemotherapy, especially with etoposide or teniposide, because of few overlapping toxicities. In addition, dose-dependent activity and even synergy has been demonstrated in animal models (Achterrath *et al.*, 1982; Chen *et al.*, 1984; Ross *et al.*, 1984; Long *et al.*, 1985).

Until now, more than 15 combination schemes for esophageal cancer have been reported. Two schedules have been studied with adequate numbers of patients: cisplatin + 5-fluoro-uracil and cisplatin + vindesine + bleomycin. This latter combination has induced substantial pulmonary toxicity, although a response rate of approximately 50% in several reports was reported (Kelsen *et al.*, 1983; Dinwoodie *et al.*, 1986; Kelsen *et al.*, 1990). Response rates of cisplatin + 5-fluoro-uracil +/-

leucovorin treatment are in the 35-50% range, sometimes even higher (Bleiberg *et al.*, 1991; De Besi *et al.*, 1986; Iizuka *et al.*, 1991; Hayashi *et al.*, 1992; Spielmann *et al.*, 1993).

In this study, we applied the same dosages and schedule of cisplatin and etoposide, as we have previously reported in patients with non-small cell lung cancer (Splinter *et al.*, 1986). The rationale of an extended administration of etoposide over several days has been justified by several authors in the light of the schedule dependent cytotoxicity of this drug (Cavalli *et al.*, 1978; Slevin *et al.*, 1989). In addition, this regimen reduces the length of hospital stay to a maximum of 3 days. The toxicity turned out to be manageable with 2 toxic deaths (3%) and 7 other patients (10%) who refused continuation because of side effects. Dose escalations of etoposide could be applied more often than dose reductions were required. The dominant side effects of nausea and vomiting (WHO 2+3: 72%), observed in our study, can presently be reduced or even eliminated using 5-HT3 receptor antagonists.

Adenocarcinoma of the esophagus is being seen increasingly frequently among Western European and American patients. In our hands however, this regimen showed no activity in patients with this histologic subtype, as previously reported (Kok *et al.*, 1988).

Our results seem to equal those of other cisplatin-based regimens. The favorable toxicity profile of our regimen has led us to perform a phase II trial of the combination of cisplatin, etoposide and a third active drug: 5-fluorouracil.

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Table 1. Patient characteristics (n=73)

male	53
female	20
age, years	
median	60
range	41 - 76
WHO performance status	
0	2
1	50
2	21
Weight loss, %	
unknown	1
0	3
1 - 5	14
6 - 10	20
10 - 20	28
> 20	7
Tumor sites	
lymph nodes	72
supraclavicular	39
mediastinal	10
coeliac	3
oesophagus	3
stomach	1

Table 1 (continued). Patient characteristics (n=73)

Tumor sites	
pleura	2
lung	5
liver	17
peritoneum	2
kidney	1
adrenal gland	1
bone	1
skin	1
Histological Type	
squamous cell carcinoma	70
undifferentiated large cell carcinoma	3
Number of organ sites	
1	45
2	16
≥ 3	12
Prior treatment (n=26)	
radiotherapy	5
surgical resection	3
radiotherapy + surgical resection	5
Celestin tube	13

Table 2. Response evalution (n=65)

Response	patients	%
Complete Response	5	8
primary + lymph nodes	4	
Partial Response	26	40
primary + lymph nodes	5	
lymph nodes	16	
liver + lung	5	
Stable Disease	22	34
Progressive Disease	9	14
Early Progressive Disease	3	4

Table 3. Haematologic toxicity

WHO (71 patients)	0	1	2	3	4	3+4 (%)
hemoglobin	17	21	30	3	0	4,2
WBC	11	10	22	19	9	39,4
platelets	38	8	8	12	5	23,9

WHO (218 cycles)	0	1	2	3	4	3+4 (%)
hemoglobin	93	85	38	3	0	1,4
WBC	57	45	71	36	9	20,6
platelets	144	17	25	23	7	13,9

Table 4. Non-haematologic toxicity

WHO	0	1	2	3	4
nausea/vomiting (71 pts)	6	13	25	26	1
alopecia (66 pts)	2	1	19	38	6
diarrhea (71 pts)	61	3	7	0	0
infection (71 pts)	55	2	6	6	2
peripheral neuropathy (71 pts)	67	2	1	1	0
renal (71 pts)	31	36	4	0	0

WHO	0	1	2	3	4
nausea/vomiting (243 cycles)	35	59	102	46	1
alopecia (225 cycles)	7	5	90	113	10
diarrhea (250 cycles)	237	5	8	0	0
infection (250 cycles)	228	2	12	6	2
peripheral neuropathy (250 cycles)	243	2	4	1	0
renal (243 cycles)	173	66	4	0	0

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CHAPTER 3

5-FLUORO-URACIL AND FOLINIC ACID IN ADENOCARCINOMA OF THE ESOPHAGUS OR ESOPHAGO-GASTRIC JUNCTION AREA

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Annals of Oncology (1996) 7, 533-534

SUMMARY

Background: The results of chemotherapy against gastric cancer or esophageal squamous cell carcinomas cannot be generalized to adenocarcinomas of the esophagus. Therefore the combination of 5-fluorouracil and folinic acid was studied in esophageal adenocarcinoma.

Patients and Methods: After a loading dose of 4 x 90 mg folinic acid orally, a continous infusion of 5-fluorouracil 500 mg/sqm/day for 5 days with concommitant folinic acid 6 x 60 mg/day orally, was administered to 29 consecutive patients with metastatic adenocarcinoma of the esophagus or esophagogastric junction area.

Results: This schedule was tolerated well with mild mucositis and diarrhea. In one patient reversible cardiotoxicity was seen. Five patients obtained a partial remmission (19%; 95% confidence interval: 6-38%), and 8 patients stable disease. One early death.

Conclusions: This combination has modest activity against adenocarcinoma of the esophagus; its toxicity profile permits incorporation in combination protocols.

INTRODUCTION

Adenocarcinoma is the predominant cell type in most patients with cancer of the esophagogastric junction area. It can be found in 5-10% of patients with cancer of the esophagus. The prognosis is poor; distant relapse is more common than local recurrence after intentionally curative resections.[1] There are very few data of chemotherapy trials designed especially for this tumorgroup; most studies consider these tumors as gastric

cancers, with corresponding treatment protocols incorporating 5-fluoro-uracil (5-FU). The addition of folinic acid (FA) to 5-FU resulted in higher response rates in gastric cancer.[2] The key determinant of potentiation of 5-FU action by FA seems to be a pronounced and prolonged inhibition of thymidylate synthetase (TS).[3] After repetitive oral administration of FA, high plasma levels of reduced folates can be achieved.[4] We investigated 5-FU, biochemically modulated with high dose FA in patients with metastatic adenocarcinoma of the esophagus or esophagogastric junction area.

PATIENTS AND METHODS

All patients were required to have histologically confirmed adenocarcinoma of the esophagus or esophagogastric junction area; no prior chemotherapy; measurable tumor parameters; performance status (WHO) 0-2; life expectancy > 3 months; adequate bone marrow- and kidney function.

Treatment consisted of FA 90 mg, given orally every hour for 4 times, followed by a continuous infusion of 5-FU 500 mg/sqm-/day for 5 days. During the 5-FU infusion, 60 mg of FA was given orally every 4 hours for a total of 30 doses. Courses were repeated every 4 weeks. In case of mucositis grade ≥ 3 (WHO), diarrhea grade ≥ 3, cutaneous toxicity grade ≥ 3, or bone marrow toxicity grade ≥ 2, the dose of 5-FU was reduced to 80% in the following courses. Domperidon was given as standard anti-emetic drug. Response evaluation was done after every 2 courses according to WHO criteria. Patients were treated up to 6 courses or until progression.

RESULTS

One patient died suddenly 10 days after his 1st cycle. Another patient discontinued treatment after his 1st cycle, because of a poor general condition. The remaining 27 evaluable patients were treated with a median of 2 to 3 cycles. Patient characteristics are shown in table 1.

No myelosuppression was seen. No hand-foot syndrome or CNS toxicity was encountered. Transient vomiting (grade 2) occurred in 11% of all cycles. In 4 patients dose reductions of 5-FU in the 2nd and subsequent courses were carried through, because of mucositis grade 3 (n=2), diarrhea grade 3 (n=1), and cardiac toxicity grade 2 (angina pectoris with transient EKG abnormalities) in 1 patient. Treatment was discontinued because of stable disease after more than 2 cycles with subsequent patient refusal (n=2), progression (n=19), or end of protocol (n=6). Toxicity data are shown in table 2.

In five patients (19%; 95% confidence interval: 6-38%), a partial remission could be seen with response durations of resp. 2 (n=2), 4 (n=2), and 6 months (n=1). Eight patients (29%) achieved stabilisation of their disease, and in 14 patients (52%) the tumor progressed during treatment. Two responding patients were treated afterwards with radiotherapy (survival from start of chemotherapy 8.7 and 19.6 months), and one responding patient underwent an esophageal resection (survival 18.8 months). The median survival of all patients was 6,5 months (range 0.3-25.9 months).

DISCUSSION

Until now, there have been no reports on single agent 5-FU treatment with FA in patients with adenocarcinoma of the esophagus or esophago-gastric junction area. After the initial publication of Machover in 1986, reporting a 48% objective

response rate in 26 patients with advanced gastric cancer, with a 3-weekly regimen of bolus 5-FU and concurrent FA,[5] several studies have been initiated to confirm these results in gastric cancer patients.[6] [7] [8] Overall, the partial response rate seems to range from 8 to 13% in first line treatment.
In our study in patients with adenocarcinoma of the esophagus, a prolonged infusion of 5-FU, biochemically modulated with orally given FA resulted in a 19% response rate. In a pilot phase, a higher dose of 5-FU (750 mg/sqm/day for 5 days) with the same FA schedule resulted in severe mucositis and moist cutaneous desquamation in 2 patients; a lower dose of 5-FU (500 mg/sqm/day for 5 days) was tolerated well by the same patients, ruling out a possible congenital deficit of dihydropyrimidine dehydrogenase (DPD). Reversible cardiotoxicity was documented in one patient. With regard to the toxicity profile, combinations with other agents, like cisplatin, and/or paclitaxel seem to be possible.

Table 1. Characteristics of patients (n=29)

Sex		
male	25	
female	4	
Median age (yrs)	60	(41-74 yrs)
WHO performance status	1	(range 0-2)
Weight loss, %		
unknown	2	
0	1	
1-5	9	
6-10	9	
11-20	6	
> 20	2	
Extent of disease		
metastatic	8	
locoregional and metastatic	21	
Localisation of tumour sites		
lymph nodes	17	
lungs	4	
liver	14	
intra-abdominal	1	
other	4	

Table 2. Non-haematologic toxicity (n=29)

WHO	0	1	2	3	4
nausea/vomiting	8	18	3	0	0
oral mucositis	9	5	10	5	0
diarrhea	20	2	3	4	0
hair	12	14	2	1	0
cardiac	28	0	1	0	0

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CHAPTER 4

IFOSFAMIDE IN ADENOCARCINOMA OF THE ESOPHAGUS OR ESOPHAGO-GASTRIC JUNCTION AREA

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SUMMARY

Twenty five patients with inoperable or metastatic adenocarcinoma of the esophagus or esophageal-gastric junction area were treated with Ifosfamide 6 grams/m^2 over 48 hours with Mesna 6 grams/m^2. One CR and 1 PR were seen among 23 patients evaluable for response, with a response duration of resp. 24 months and 7 months. Toxicity was mild: grade 3 infection in 1 patient (urosepsis, leucocyte nadir 1,7) grade 3 leucopenia in 4 patients, grade 3 nausea in 4 patients. No life threatening episodes, nor CNS toxicity were encountered. Ifosfamide has limited but definite activity in adenocarcinoma of the esophageal-gastric junction area.

INTRODUCTION

The outlook for patients with adenocarcinoma of the esophagus is dismal; in about 40% metastatic disease is apparent at first presentation. Even if a patient is operable, the five years survival after surgery with curative intent is <10%. Most of these patients die with distant metastases. In this way, there is a clear need for new effective chemotherapy. In a series of phase II studies concerning this tumortype we investigated the activity and toxicity of Ifosfamide.

PATIENTS AND METHODS

Until July 1990, 25 consecutive patients were entered in the study. The main eligibility criteria were: histologically proven adenocarcinoma of the esophagus or esophago-gastric junction area, with or without Barrett's epithelium. Patients with adenocarcinoma of the gastric cardia, without involvement of the esophageal-gastric junction area are not eligible;

performance status (Karnofsky) >60% and a life expectancy of ≥ 3 months; age ≤75 years; no prior chemotherapy; no symptomatic brain metastases; adequate bone marrow- and kidney function (clearance >60ml/min); measurable lesions - only if the primary tumor was the only marker lesion, and not previously irradiated, the disease was considered evaluable and monitored by barium radiogram, CT-scan and endoscopy with biopsies; a garanteed good intake (in cases of severe stenosis, a tube was inserted). Patients with a probability of <0.05 of not developing severe CNS toxicity after Ifosfamide/Mesna infusion, calculated by means of serum albumin and creatinine concentration according to the monogram described by Meanweel et al.(1), were not eligible.
Treatment consisted of prehydration with 500 ml saline 0.9% for 2 hrs followed by 200 ml Mannitol 20% over 30 minutes and Mesna 1 gr/m^2 i.v. Ifosfamide was given as a 48 hours infusion in a total dose of 6 gr/m^2 together with 6000 ml dextrose/saline and Mesna 3 gr/m^2. Posthydration was given with 2500 ml dextrose/saline over 16 hours together with Mesna 2 gr/m^2. Courses were repeated after 4 weeks. Patients were evaluable for response after 2 courses, and evaluable for toxicity after one course. In cases of clear progression after the first course, the response was evaluated as early progression. In cases of stable disease after any two cycles, treatment was stopped. The maximal duration of treatment was 6 cycles or until progression of disease or intolerable toxicity, physically or mentally. The recommended guidelines for the criteria of evaluation and toxic effects proposed by the WHO (1979) were followed.

RESULTS

Of 25 entered patients, one was ineligible (adenocarcinoma of the gastric cardia without involvement of the esophageal-

gastric junction area) and one was inevaluable for response (no response evaluation after the 2nd course). The main characteristics of 23 evaluable patients and their tumors are shown in table 1 and 2. The majority of patients were men with a fair performance status, not withstanding a significant weight loss, who had metastatic cancer at first presentation.

Toxicity data of 24 patients are shown in tabel 3, graded according WHO 1979. Of a total of 63 courses, the median number was only two. There was no treatment delay because of cytopenia; only one time a leucocyte nadir of 1.1 in combination with fever required a dose reduction of 25%. The median WBC nadir was 4.0 (1.1-6.3) x 10^9/L, the median platelet nadir 262 (174-571) x 10^9/L. In two cases a serious infection (WHO 3) required hospital admission for intravenous antibiotic therapy (WBC nadir 1.7 and 1.1 x 10^9/L). No toxic death. No serious renal, nor any CNS toxicity were seen during the study. Reasons for going off study were: serious subjective toxicity (n=1), progressive disease during treatment (n=8), stable disease after two courses (n=7), end of protocol (six courses, n=4). One patient experienced rapid deterioration of his condition after the first course: no response evaluation was done.

Among 23 patients evaluable for response, one achieved a CR (response duration 26+ months [Nov.1990]) and one a PR (response duration 7 months).

The first patient, male 47 years, with a poorly differentiated adenocarcinoma in Barrett's epithelium and microscopically proven metastatic lymph nodes in the coeliac region, had a clinical complete remission after 4 courses. He refused surgical treatment after his chemotherapy, and is now, two years later, in perfect condition without clinically detectable tumor (endoscopy + biopsies).

The second patient, male 60 years, with microscopically proven metastatic cancer in the coeliac and supraclavicular lymph nodes had a partial remission after 6 courses. No

supraclavicular nodes (ultrasound) were detectable any more, but the coeliac nodes were still somewhat enlarged; a small ulcer was the remnant of his primary tumor, biopsies were negative. Weight gain during chemotherapy was 13.5 kg. Two months after the 6^{th} course brain metastases and rapidly progressive malignant lymfangitis in both lungs appeared.

The median survival time of 21 deceased patients (Nov. 1990) was 7 months (2-54) after diagnosis, 3.5 months after stopping chemotherapy, and 6 months after starting chemotherapy. Three patients are still alive, with a follow-up of resp. 2, 3 and 24 months after stopping Ifosfamide.

DISCUSSION

Although 2 patients in this phase II study achieved a well documented major regression, Ifosfamide seems to have a low activity in untreated patients with advanced adenocarcinoma of the esophagus or esophageal-gastric junction area. The lack of response is confirmed by several other trials (3,4). However,we could not confirm the severe toxicity described in these reports. Some factors could play a role in this discrepancy: our patients had a better performance status than those described by Ansell. More than half of the patients in Kelsen's report were pretreated with radiotherapy and/or chemotherapy. Concerning the dose and schedule of Ifosfamide we delivered 6 gr/m^2 as a continuous infusion over 48 hours instead of 7.5 gr/m^2 over 5 days as daily short i.v. infusions. On the other hand our bone marrow data are not different from those of Ansell, and clearly less serious than those of Kelsen et al.

In conclusion, Ifosfamide has a low efficacy as first line chemotherapy in patients with adenocarcinoma of the esophagus. The application of a continuous administration over several

days may be beneficial regarding the serious toxicity described in reports using a fractionated regime with short i.v. infusions for several days.

Table 1. Characteristics of 23 evaluable patients

Male / Female	21 / 2
Median age (yr)	55 (30-74)
Median performance status	80 (60-100)%
Extent of disease	
Locally advanced	2
Primary excised, metastases	4
Intra-abdominal	1
Liver	1
Pleural	1
Skin	1
Primary plus metastases	
Intra-abdominal	2
Liver	6
Lymph nodes	9
Median weight loss	10 %
< 1 %	1
1 - 5 %	4
6 - 10 %	7
> 10 %	11

Table 2. Tumour characteristics

Adenocarcinoma in Barrett's epithelium	4
Signet-ring in Barrett's epithelium	2
Garde of malignancy (ICD-O)	
grade 2	15
grade 3	7
unknown	1
Location of primary tumour	
Lower oesophagus with junction area	9
Lower oesophagus with junction artea and cardia	10
Junction area plus cardia	2
Unknown	2

Table 3. Toxicity profile (n=24)

Total courses	63
Median courses	2 (1-6)
Dose reduction	1 x
Treatment delay (cytopenia)	0 x
Median WBC nadir	4.0 (1.1-6.3) x 10^9/l
Median platelet nadir	262 (174-571) x 10^9/l
Toxic death	0

	WHO grades				
	0	I	II	III	IV
WBC	13	2	6	3	
Infection	22			2	
Platelets	24				
Nausea, vomiting	1	6	13	4	
Hair		3	15	6	
Neurotoxicity	24				
Renal	23	1			
Cutaneous	23	1			

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CHAPTER 5

13-*CIS*-RETINOIC ACID AND ALPHA-INTERFERON IN ADVANCED SQUAMOUS CELL CANCER OF THE ESOPHAGUS

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INTRODUCTION.

Retinoids, a class of compounds related to vitamin A, normally play a role in growth, vision, epithelial cell differentiation, and immune function.[1] A preventive effect of vitamin A on the development of chemically induced tumor has been demonstrated, as well as a therapeutic effect in cancer.[2] Cyto-kines, such as interferons, demonstrate a synergistic effect with retinoids on the inhibition of proliferation in squamous cell carcinomas of the cervix, head and neck, and skin.[3] Here we report the results of a phase II study of 13-*cis* retinoic acid plus interferon alpha-2A in patients with metastatic squamous cell carcinoma of the esophagus.

PATIENTS AND METHODS

All patients were required to have measurable disease; age ≤ 75 years; no prior chemotherapy; performance status (WHO) 0-2; life expectancy > 3 months. Treatment consisted of Interferon alpha-2A (IFN-α, Roferon-A^R, Roche) subcutaneously $3x10^6$ IU/-day, plus 13-*cis*-retinoic acid (cRA, isotretinoin, Roaccutan^R, Roche) orally 1 mg/kg/day. Treatment was continued for at least 2 months in all patients, unless disease progressed earlier, and at least 3 months in case of no change, unless toxicity was intolerable. Response and toxicity evaluations were done according to WHO criteria.

Ten patients entered the study, all evaluable. The median duration of treatment was 8 weeks (range: 4-33 weeks). One patient discontinued treatment after 4 weeks because of overt progressive disease. The patient characteristics are shown in table 1.

RESULTS

No or very mild nausea (WHO grade 0,1) was present in 9 patients, grade 2 in 1 patient. Two patients developed grade 1 leucopenia, and nearly all patients showed a slight but discernable decrease in platelets, but still within the normal range (WHO 0). In one patient the dose of cRA was reduced to 0.5 mg/kg/day because of difficulty in swallowing, and a dry and moderately painful skin. A dry skin was noted in 8 patients (WHO 1). Fatigue was seen only during the first 1 or 2 weeks after start of treatment. No elevations of serum transaminases could be detected.

Treatment was discontinued because of progression (n=8), or poor general performance status (n=2).

No objective responses were seen. Two patients had stable disease for a duration of 2 and 10 months. All patients have died. The median survival was 8.8 months (range 3.6 - 14.9 months) after start of treatment.

In this study, no objective responses to the combination of cRA and IFN-α were seen (95% confidence interval: 0-31%). One patient experienced stabilisation of the disease for a period of 10 months. The toxicity of this regimen was mild. Recent clinical studies in squamous cell cancer of the skin and cervix have demonstrated greater antitumor activity of the combination of cRA and IFN-α, compared with either agent alone, [4] [5] although 2 phase II studies in advanced non-small-cell lung cancer could not confirm these results: 2 PR's in 58 patients.[6] [7] Toma et al reported on 2 patients with esophageal cancer ; both achieved complete remission after treatment with IFN-α $6x10^6$ IU/day and cRA 1 mg/kg/day with a response duration of 8 and 36 months respectively.[8] In our study the IFN-α dose was $3x10^6$ IU/day, and a second difference may be the stage of disease; 7 of our patients had bulky disease at the start of treatment with the primary tumor still in situ.

Table 1. Patient characteristics (n=10)

Sex		
male	6	
female	4	
Median age (yrs)	59	(35-73 yrs)
WHO performance status	1	(range 0-2)
Weight loss, %		
unknown	2	
1-5	2	
6-10	3	
11-20	3	
Extent of disease		
metastatic	3	
locoregional and metastatic	7	
Localisation of tumor sites		
lymph nodes	11	
lungs	2	
liver	5	
other	1	
Pretreatment		
Esophageal resection	3	

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Ann Oncol 1994, 5,463-465.

CHAPTER 6

NEO-ADJUVANT CHEMOTHERAPY COMPARED WITH SURGERY IN ESOPHAGEAL SQUAMOUS CELL CANCER

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Submitted

ABSTRACT

Background Although several studies suggest an improvement on survival of patients with esophageal squamous cell cancer after combined preoperative chemotherapy and radiotherapy, the exact role of chemotherapy is not clear. We conducted a multicentre prospective, randomized trial comparing preoperative chemotherapy plus surgery with surgery alone.

Methods Patients assigned to preoperative treatment received two courses of chemotherapy in weeks 1 and 4 (cisplatin 80 mg per square meter of body-surface area on day 1, and etoposide, 100 mg iv on day 1 and 2, and 200 mg per square meter orally on day 3 and 5). In case of a major clinical response, another 2 courses of the same chemotherapy were given in weeks 8 and 11, followed by surgery by means of a transhiatal esophageal resection, whereas non-responding patients were referred to surgery at once. Patients assigned to surgery alone had no preoperative treatment.

Results Four of 85 patients assigned to preoperative chemotherapy, and 2 of 84 patients assigned to surgery alone were excluded from analysis because of ineligibility. A clinical response after chemotherapy could be evaluated in 76 patients: 23 had a major regression, and in 6 patients the primary tumor disappeared completely. Of 81 patients assigned to chemotherapy, 67 underwent surgical resection, versus 69 of 82 patients assigned to surgery alone. In four patients no infiltrating tumor was found in the resection specimen after chemotherapy. Fifty-nine of 66 patients (89%) had a resection with tumour-free margins after chemotherapy, as compared with 37 of 68 patients (54%) after surgery alone ($P < 0.001$). Median survival of all patients assigned to chemotherapy was 19 months, versus 12 months for those assigned to surgery alone ($P = 0.003$). At one, two, and three years, 69, 48, and 41 percent, respec-

tively, of all patients assigned to chemotherapy were alive, versus 48, 30, and 21 percent of those assigned to surgery alone.

Conclusions Preoperative chemotherapy in patients with resectable squamous cell carcinoma of the esophagus prolongs survival, as compared to surgery alone.

INTRODUCTION

The outlook for patients with esophageal cancer is poor. Even in patients with localized disease, the results after surgery or radiotherapy are not satisfactory, with a median survival of one year or less, and a five-year survival rate of only 10 percent.(1)·(2)·(3) In the Western population, where locally advanced tumors are frequently observed, most patients have subclinical metastases at presentation.(4) Postoperative combination chemotherapy after primary local treatment has not been successful yet, and is poorly tolerated for a considerable time after esophageal surgery. Preoperative chemotherapy and/or radiotherapy have been attempted to reduce the size of the primary tumor and improve the resectability rate, and eliminate subclinical metastases. Indeed, several randomized studies have already shown that a complete pathological response rate of approximately 20 percent can be achieved after multi-modal therapy, with a survival advantage over patients treated with surgery alone.[3]·(5) However, the relative contributions of preoperative chemotherapy and radiotherapy to a survival advantage are unclear and concurrent application of radiation and intensive chemotherapy can lead to substantial toxicity.(6)·(7) We therefore designed a randomized trial to determine whether preoperative chemotherapy plus surgery was superior to surgery alone in patients with squamous cell carcinoma of the esophagus.

METHODS

Patients

All patients who met each of the following criteria were eligible for the study: age below 80 years, histologic evidence of invasive squamous-cell- or undifferentiated non small cell carcinoma of the thoracic esophagus without gastric involvement, a priori resectable for cure, without evidence of distant metastases (including supraclavicular lymph node metastases), a Karnofsky performance score of 70 or higher, life expectancy greater than 6 months, adequate calory intake, initial leukocyte count above 3.5×10^9 cells per liter, platelet count above 100×10^9 cells per liter, serum creatinine concentration less than 130 μmol per liter (1.4 mg per deciliter) or creatinine clearance above 60 ml per minute, adequate pulmonary function, no previous malignancy except basal cell cancer of the skin or adequately treated carcinoma in situ of the cervix, no previous chemotherapy or radiotherapy, no uncontrolled heart failure , hypotension, or significant arrythmia. Patients with an upper thoracic or cervical carcinoma, requiring laryngectomy, and patients with an esophageal tube or stent in situ or with an otherwise inevaluable tumour were not eligible. The study protocol was approved by the Ethics Committee of all participating institutions, and by the Review Board of the Netherlands Cancer Foundation. Informed consent was obtained from all patients, included in this study.

Tumor Staging and Evaluation.

In all patients the disease was staged by physical examination, chest radiography, ultrasonography of the cervical and upper abdominal region, upper gastrointestinal endoscopy with biopsies, and computed tomography of the chest and upper abdomen. Sometimes a barium esophagography and bronchoscopy were performed, the latter if indicated by the location of the tumor or symptoms. Radionuclide bone scans were performed only if indicated. The clinical response after chemotherapy was

evaluated by esophagoscopy, computed tomography of the chest, and by esophagography. The complete absence of any evidence of malignant disease including negative biopsies from the former tumor area was defined as Complete Response (CR). A more than 50% reduction in tumor bulk, but residual disease still evident, without the appearance of new lesions was defined as Partial Response (PR). Stable Disease (SD) was defined as neither evidence of progression, nor > 50 percent reduction of tumor bulk, without the appearance of new lesions. More than 25 percent progression of tumor bulk, or the appearance of a new lesion was defined as Progressive Disease (PD). The tumor stage after resection was defined according to the TNM classification of the International Union Against Cancer (UICC).(8)

If no residual tumor was detected, the disease was classified as a pathological complete response. The disease was classified as stage 1 (pT_1N_0) if tumor was found in the mucosa or submucosa without involvement of the regional lymph nodes, and as stage 2A, if tumor was found, infiltrating (pT_2) or penetrating (pT_3) the muscular layer of the esophagus without regional lymph node metastases. If also lymph nodes metastases were found, the disease was defined as stage 2B. Stage 3 was restricted for a tumor with deep infiltration beyond the esophageal wall with regional lymph nodes metastases (pT_3N_0) or for a tumor infiltrating surrounding organs with or without regional lymph node metastases (pT_4N_{any}). If distant metastases were detected, the disease was classified as stage 4. To describe the absence or presence of residual tumor after resection of the primary, the following R(esidual) categories were used as appendices: R_x if presence of residual tumor could not be assessed, R_0 if all the surgical margins were free of tumor, R_1 if there was microscopic residual tumor in any of the surgical margins, and R_2 if macroscopic residual tumor was detected.

Study Design.

After informed consent, patients were initially stratified for gender, age, weight loss in the past 4 months and largest dimension of the tumor as detected by esophagoscopy. Randomization took place subsequently between chemotherapy followed by surgery or surgery alone. Patients assigned to preoperative chemotherapy were treated with two courses, followed by a clinical response evaluation. In case of a major response (CR or PR), two extra courses of the same chemotherapy were administered, followed by surgery, whereas non-responding patients (SD or PD) were referred for surgery at once. Patients were followed until death at three months intervals. At each visit during follow up a physical examination and liver biochemistry was performed. Ultrasonography of the cervical and upper abdominal regions and esophagoscopy were performed at 6 months intervals. Other diagnostic tests were performed only when indicated.

Chemotherapy.

Chemotherapy consisted of courses of cisplatin and etoposide .(9) After 16 hours of prehydration cisplatin (80 mg per square meter of body-surface area) was infused over 4 hours on day 1, followed by 24 hours of posthydration. Etoposide 100 mg was administered intravenously over 2 hours on day 1 (before cisplatin) and 2. On day 3 and 5 etoposide 200 mg per square meter of body-surface area was taken orally at home. In case of difficulties in swallowing etoposide capsules, the contents were diluted in syrup. This course was repeated in week 4. To counterbalance possible differences in bioavailability of oral etoposide, the oral dosages were modified individually to reach a leukocyte nadir between 1.0 and 2.0 x 10^9 cells per liter and a platelet nadir between 25 and 100 x 10^9 cells per liter. In the event of a responding tumor, 2 subsequent courses of chemotherapy were administered in week 8 and 11. All patients were treated with 5-hydroxytryptamine$_3$ receptor

antagonists prophylactically during chemotherapy.

Surgery.

Surgery was carried out as soon as possible after randomization for patients assigned to surgery alone, or between 4 and 6 weeks after the last chemotherapy course, provided that the leukocyte count was more than 3.0 x 10^9 cells per liter, and the platelet count was more than 100 X 10^9 cells per liter. After laparotomy the abdominal cavity was inspected for metastases, with special attention to the liver and the celiac region. Resection was discarded if metastases could be proven histologically. Thereafter the resectability of the primary tumor was considered. A subtotal transhiatal esophagectomy was performed, without thoracotomy. The continuity of the digestive tract was restored by a stomach tube reconstruction or colonic interposition procedure with anastomosis in the neck.

Chemotherapy-induced toxicity.

All supportive measures consistent with optimal patient care were given. The World Health Organization (WHO) recommendations for grading of toxic effects of chemotherapy were followed.(10) A subsequent chemotherapy course was delayed,- if the leukocyte count on the first day was below 3.5 x 10^9 cells per liter, and/or the platelet count below 100 x 10^9 cells per liter. Chemotherapy was stopped permanently if the counts did not recover within 2 weeks. In case of renal toxicity WHO grade 3 or 4, or neurotoxicity WHO grade 3 or 4, chemotherapy was stopped. If chemotherapy was stopped for toxicity reasons, patients were operated as soon as possible.

Statistical Analysis.

The planned number of patients to be entered in the study was 80 for each treatment arm. With these numbers of patients the statistical power should be sufficient (beta = 0.20; alfa 0.05) to detect an increase of the median survival from 10 to

18 months. A committee of independent experts monitored the progress of the trial, including an assesment of toxicity in patients assigned to chemotherapy. The number of planned interim evaluations during the trial was three. Survival was measured from the date of randomization to the date of death or most recent follow-up visit. Time to distant metastasis and time to local recurrence after radical (R0) resection were measured from the date of randomization and resection respectively, to the occurrence of either event or most recent follow-up visit. Estimates of survival and time to disease recurrence were based on the Kaplan-Meier method, group comparisons were based on the log-rank test. The chi-square or Fisher's exact test was used for comparison of categorical data.(11)

RESULTS

Demographic Data

Between March 1989 and October 1996, 169 patients were enrolled in the study. Eighty-five patients were randomly assigned to receive chemotherapy before surgery, and 84 patients were assigned to surgery alone. Six patients (4 patients assigned to chemotherapy and 2 patients assigned to surgery alone) were excluded from the analysis. The reasons for exclusion are listed in Table 1. The characteristics of 163 fully eligible patients at the time of randomization are listed in Table 2.

At the census date the median duration of follow-up for all patients was 3.8 years.

Protocol Compliance

Three patients refused chemotherapy after randomization and underwent primary surgery. Four patients were operated after the first course of chemotherapy, 3 because of progressive disease, and 1 because of grade III renal toxicity. These 7 patients were included in the chemotherapy group analysis.

None of the patients refused surgery after chemotherapy.

One patient assigned to surgery alone could not be operated because of a myocardial infarction just before his planned surgery. This patient was included in the surgery alone group analysis.

Surgery took place at a median of 14 days (range, 4 to 60) after assignment to surgery alone. In the chemotherapy group, the median duration from randomization to surgery for non-responding and responding patients was 2.1 months (range, 1.2 to 4.1) and 3.8 months (range, 1.8 to 5.5) respectively.

Treatment-Related Toxicity

One patient had a fatal neutropenic sepsis after chemotherapy. Twenty-three patients (28 percent) experienced grade III toxic reactions as defined by WHO criteria (one renal, twenty-two hematologic), and 8 other patients (10 percent) grade IV (all hematologic). Alopecia was common.

There was no difference in surgical complications and postoperative morbidity and mortality between the two groups. The postoperative 30-day mortality rate was 4 percent after surgery alone and 3 percent after chemotherapy plus surgery. The median duration from the day of resection until discharge was 17 days in the preoperative chemotherapy group and 16 days in the surgery alone group.

Clinical Response to Chemotherapy

Seventy-six of 81 patients assigned to preoperative chemotherapy could be evaluated clinically for response to chemotherapy; three patients declined chemotherapy after randomization, and in two patients chemotherapy had to be stopped after the first course for toxicity reasons. Six patients achieved a CR and 23 patients a PR, resulting in a 38 percent overall response rate. Thirty four patients (45 percent) had SD, and 13 patients (17 percent) had PD; four of these de-

veloped celiac lymph node metastases and were not operated. Of the remaining 9 patients with local-regional progression, one received external radiotherapy, and two others were treated with endoscopic intubation for a broncho-esophageal fistula. Six patients with local progression were referred for surgery; three had an irradical resection (R_1 or R_2), and in 3 patients the tumor was not resectable at all.

Results at Surgery

Resection rates were similar in the 2 groups: 83 percent in the preoperative chemotherapy group, and 84 percent in the surgery alone group. In the chemotherapy group, 7 patients were not operated, mainly because of progressive disease, and in 7 other patients the tumor was not resected because of encasement of the aorta or the bronchial tree, or the detection of metastatic celiac lymph nodes at laparotomy.

In the surgery alone group, 1 patient could not be operated for reasons unrelated to disease or treatment. In 12 patients the tumor was not resected because of infiltration in the aorta or bronchial tree, or the detection of distant metastatic lymph nodes at laparotomy. Table 3 lists the results at the time of surgery.

Pathology after resection

Pathology findings after resection are listed in table 4. More early T-stages and less tumors infiltrating in surrounding organs were found in the preoperative chemotherapy group. There was a significant difference in the amount of radicality between the two groups: a R_0 resection could be completed in 59 of 66 patients (89 percent) after chemotherapy, and in only 37 of 68 patients (54 percent) without preoperative chemotherapy ($P < 0.001$). Pathological examination of the resected specimen after a transhiatal approach, as performed in this trial, does not permit a reliable determination of the pN category; however a UICC stage classification was formed on the basis of

surgical and pathological data.

Survival

The median duration of follow-up for both groups was 15 months (range, 0.2 to 91). At the census date, 31 of 81 patients assigned to preoperative chemotherapy were still alive, as compared to 19 of 82 patients assigned to surgery alone. Figure 1 shows a survival advantage for patients assigned to preoperative chemotherapy: median survival time 19 months as compared with 12 months in the group treated with surgery alone. Likewise, the chemotherapy patients had a better one, two and three year survival (± S.E.) of 69 (± 5), 48 (± 6), and 41 (± 6) percent, as compared to 48 (± 6), 30 (± 5), and 21 (± 5) percent of patients assigned to surgery alone. When survival was calculated from the time of resection, the median survival time after chemotherapy plus resection was 26 months as compared to 15 months after resection alone (P = 0.01). Figure 2 shows a median survival time for patients with a major clinical response (CR,PR) after chemotherapy of 44 months, as compared to 13 months for those who did not respond. In addition, as shown in Figure 3, the rate of local-regional recurrences (± S.E.) in the chemotherapy group at 1, 2, and 3 years after radical (R0) resection was 8 (± 4), 15 (± 6), and 29 (± 9) percent, as compared to 32 (± 9), 52 (± 11), and 57 (± 11) percent for the surgery alone group. At 1, 2, and 3 years after randomization, the rate of distant metastases (± S.E.) was 26 (± 6), 29 (± 6), and 29 (± 6) percent of patients assigned to preoperative chemotherapy, as compared to 39 (± 7), 50 (± 8), and 55 (± 9) percent of patients assigned to surgery alone (Fig.4.).

DISCUSSION

This is the first randomized study in patients with squamous cell carcinoma of the esophagus with a significant survival

advantage after chemotherapy plus surgery, as compared to surgery alone. At three years the rate of survival in patients treated with preoperative chemotherapy (41%) was almost twice that of surgery only treated patients (21%). The median and long term survival in patients with tumors responding to chemotherapy was highly superior to that of non-responding patients and patients after surgery alone. Tumor free margins were found in 89% of the resection specimens after preoperative chemotherapy, as compared to 54% of specimens after resection alone. Although not a major end point in this study, a beneficial effect of preoperative chemotherapy on the time to local-regional recurrences, as well as on the development of distant metastases is present. Admitting the loss of one patient on account of chemotherapy related toxicity, this neoadjuvant treatment was feasible in combination with transhiatal esophageal resection, without seriously increased perioperative morbidity or mortality.

The efforts to stage thoracic esophageal cancers in a clinically appropriate way have been hampered by the absence of endoscopic ultrasonography at the beginning of our trial. Therefore the clinical T- and N-stage were not used as stratification parameters before randomization. In the same way, postoperative UICC stage classification could be biased by a possible underestimation of regional lymph node involvement, as a result of the transhiatal approach, which was the preferred technique of the surgeons of the participating institutions.

Until now, five other comparative trials on preoperative chemotherapy in esophageal cancer have been reported, two of which are abstracts. Roth et al reported on 39 patients with operable squamous cell carcinoma, who were treated with surgery alone or with two pre- and postoperative courses of chemotherapy (cisplatin, vindesine, and bleomycin).(12) A transthoracic surgical approach was performed in the majority of patients. The median survival for both treatment groups was

9 months. Patients with responses to chemotherapy had a significant better survival (median, > 20 months) compared with either non-responders or those assigned to surgery alone. Schlag et al treated 24 patients with surgery alone (transthoracic-abdominal en-bloc resection including celiac lymph nodes) and 22 patients with 3 preoperative cycles of cisplatin and 5-fluorouracil.(13) There were two-drug related deaths-, and postoperative morbidity and mortality was higher in patients treated with preoperative chemotherapy. The median survival in both groups was 10 months; patients responding to chemotherapy had a prolonged survival (median, 13 months) as compared with non-responding patients. In a four-arm study in 187 patients, Nygaard et al did not find a survival advantage at 3 years for patients assigned to preoperative chemotherapy with cisplatin and bleomycin (3%) versus surgery alone (9%).(14) Fok et al reported results of 2 courses of preoperative chemotherapy with cisplatin and 5-fluorouracil followed by surgery versus surgery alone in 160 patients.(15) No difference in median survival was observed (13 versus 12 months). The five year survival rate in patients responding to chemotherapy was 52 percent, as compared to 10 and 11 percent in non-responders and surgery only patients, respectively. In 1997, preliminary results of the U.S. Intergroup study 113 (RTOG 89-11) were presented.(16) More than 400 patients with squamous cell (45%) or adenocarcinoma (55%) were randomized to 3 courses of chemotherapy (cisplatin and 5-fluorouracil) followed by surgery and 2 postoperative courses of the same regimen in responding patients, or operation alone. The median survival in both groups was 16 months, with a 2-year survival rate of 38 percent for patients after preoperative chemotherapy as compared to 40 percent after surgery alone.

The effect of a prolonged follow-up in this type of comparative studies is important and can be two-sided. Preoperative chemotherapy could cause a delay in the development

of clinically overt metastases, resulting in a midterm survival benefit without an increased cure rate at five years.(17)
On the other hand, Urba et al reported very recently in abstract form their updated analysis of a randomized trial, showing a trend to survival advantage for patients assigned to multimodality therapy over surgery alone, not until after a median follow-up (for survivors) of more than 5 years.(18)

Clinical response rates in patients with metastatic esophageal squamous cell cancer after cisplatin based combination chemotherapy usually are in the 35 to 55 percent range, with 10 percent or less complete responses.[9] In our study 4 of 6 patients who achieved a clinical CR after chemotherapy, actually had a pathological CR after resection. Despite this low number, preoperative chemotherapy did have a beneficial effect on local-regional control after transhiatal resection, regarding the higher rate of R0 resections and the longer period to a local recurrence, as compared to surgery alone.
In conclusion, preoperative chemotherapy offers a significant survival advantage over surgery alone in patients with localized squamous cell carcinoma of the esophagus. The evidence suggests that a favourable response after chemotherapy predicts for survival benefit.

TABLE 1. PATIENTS EXCLUDED FROM ANALYSIS

PATIENT ID.NO.	SEX/ AGE(YR)	REASON FOR EXCLUSION	TREATMENT ACTUALLY RECEIVED	FOLLOW-UP (MO)
Allocation to surgery alone				
1	M/53	Adenocarcinoma	Surgery	15
2	M/59	Died suddenly after randomization	No treatment	0.6
Allocation to chemotherapy plus surgery				
3	F/50	Lost after randomization	Unknown	Lost
4	F/37	Adenocarcinoma	chemotherapy + surgery	13
5	M/60	Cervical cancer requiring laryngectomy	chemotherapy + surgery	0.4
6	M/59	Lost for follow-up	chemotherapy	Lost

TABLE 2. CHARACTERISTICS OF PATIENTS AFTER RANDOMIZATION.

CHARACTERISTIC	CHEMOTHERAPY + SURGERY (N=81)	SURGERY ALONE (N=82)
Age (yr)		
Median	61	62
Range	35-76	37-79
	no. (%) of patients	
Sex		
Male	60 (74)	60 (73)
Female	21 (26)	22 (27)
Tumor type		
Squamous cell	78 (96)	80 (98)
Undifferentiated non-small cell	3 (3)	2 (2)
Location tumor in esophagus		
Upper and middle third	13 (16)	14 (17)
Lower third	68 (84)	68 (83)
Weight loss, last 4 months (% of normal weight)		
0-5	30 (37)	30 (37)
6-10	19 (23)	27 (33)
>10	32 (40)	25 (30)

TABLE 2. CHARACTERISTICS OF PATIENTS AFTER RANDOMIZATION. (cont'd)

CHARACTERISTIC	CHEMOTHERAPY + SURGERY (N=81)	SURGERY ALONE (N=82)
	no. of patients (%)	
Karnofsky performance scale *		
70 - 80	60 (74)	65 (79)
90 - 100	21 (26)	17 (21)
Largest tumor dimension (cm)		
1-3	15 (19)	13 (16)
4-6	36 (44)	34 (42)
7-10	24 (30)	34 (42)
> 10	6 (7)	1 (1)

* A score of 70 to 80 indicates that the patient is at least ambulatory and capable of all self-care; a score of 90 to 100 indicates that the patient is capable to carry on normal activity or has minor effects of disease.

Table 3. RESULTS AT SURGERY.

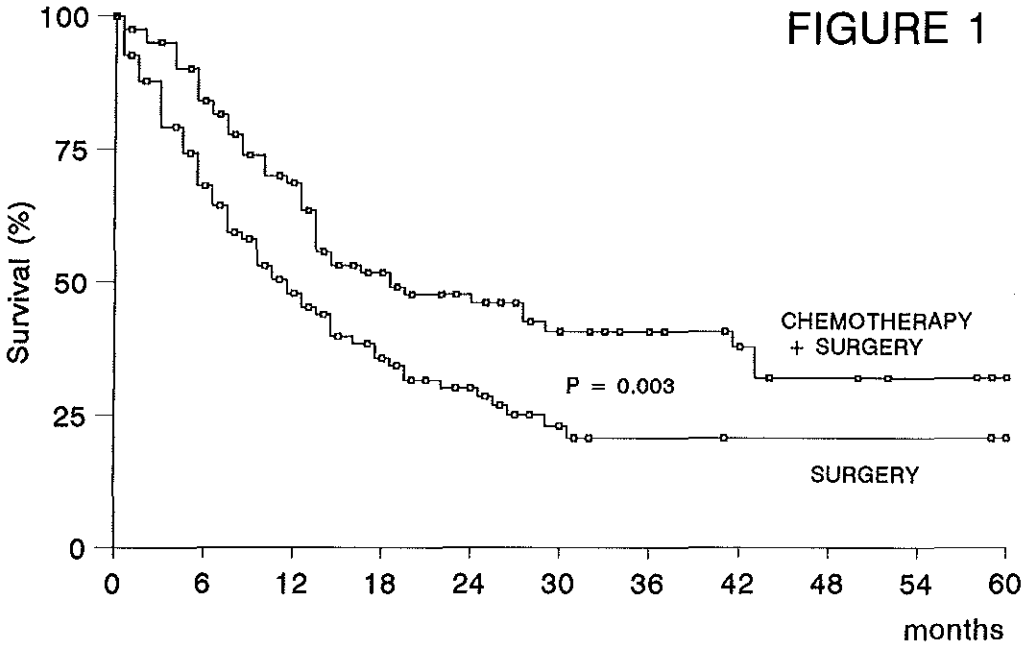
	CHEMOTHERAPY + SURGERY (N=81)	SURGERY ALONE (N=82)
NO SURGERY	7	1
Toxic death	1	
M_1 (celiac lymph nodes)	3	
Fistula	2	
Local progression	1	
Myocardial infarction		1
NO RESECTION	7	12
Regional infiltration (T_4)	6	7
M_1 (celiac lymph nodes)	1	5
RESECTIONS	67	69

Table 4. PATHOLOGY AFTER RESECTION.

	CHEMOTHERAPY + SURGERY (N=67)	SURGERY ALONE (N=69)
Unknown	1	1
UICC STAGE		
Complete Response	4	
I	7	2
IIA	26	32
IIB	1	
III	19	20
IV	9	14
PRIMARY TUMOR		
pT_0	2	
pT_{is}	2	
pT_1	8	2
pT_2	9	8
pT_3	44	52
pT_4	1	6
RESIDUAL DISEASE		
R_0	59	37
R_1	5	23
R_2	2	8

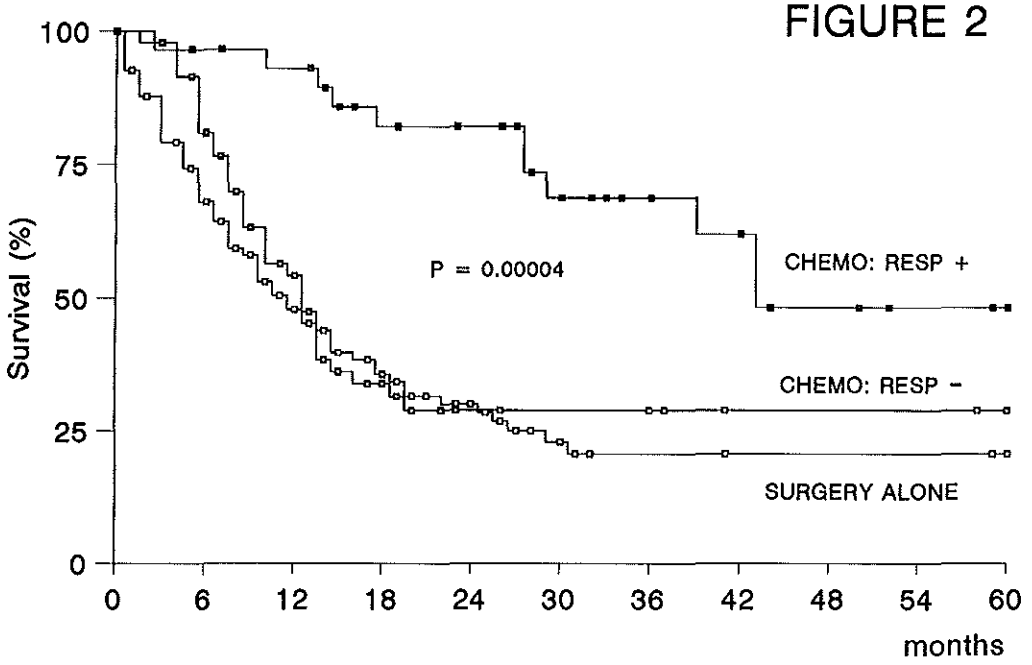
SURVIVAL FROM RANDOMIZATION

FIGURE 1



SURVIVAL FROM RANDOMIZATION

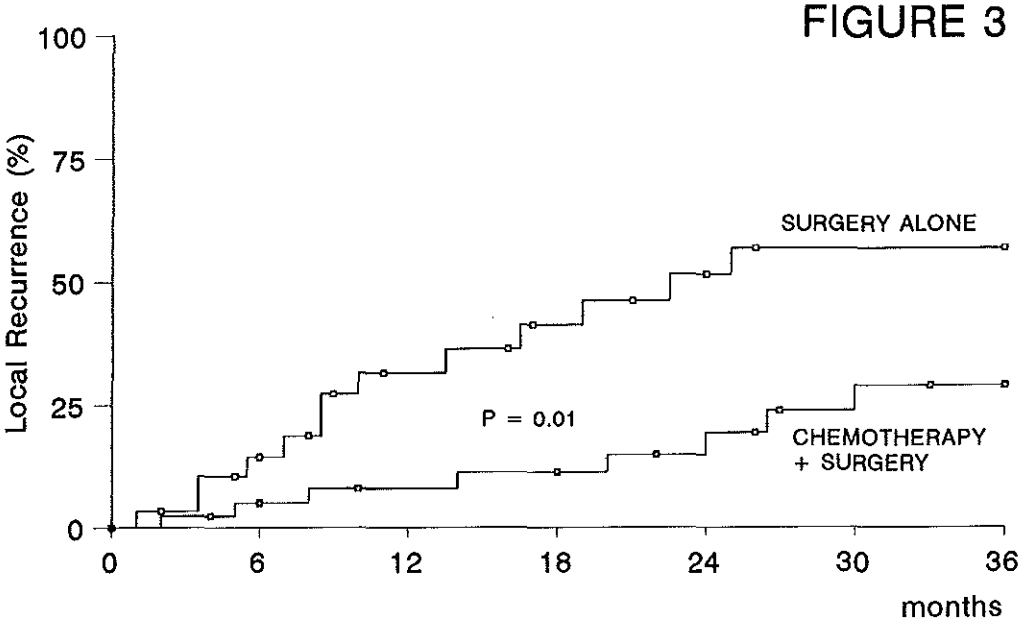
FIGURE 2



Time to Local Recurrence

After Radical (R0) Resection

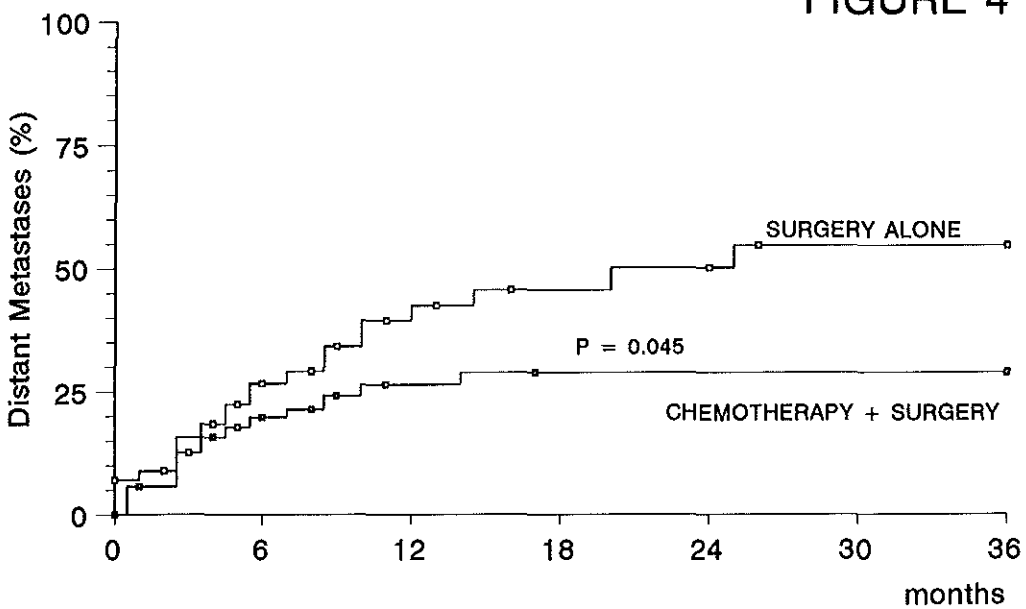
FIGURE 3



Time to Distant Metastases

After Randomization

FIGURE 4



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CHAPTER 7

SALVAGE SURGERY AFTER CHEMOTHERAPY FOR METASTATIC ESOPHAGEAL CANCER: A PILOT STUDY

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Submitted

INTRODUCTION

The prognosis for patients with esophageal cancer is still poor, even for those who undergo resection. At least in the Western population, the majority of patients has systemic disease at presentation. Ultrasonography and computed tomography of the cervical and upper abdominal area have become of great value as staging procedures, especially regarding lymph node metastases, which, according to the UICC TNM classification system, have to be considered as distant metastases in cancers of the thoracic esophagus.(1)'(2) Usually, patients with metastatic disease at presentation will be treated palliatively with intubation, radiotherapy, or sometimes, in the framework of research protocols, with chemotherapy. We studied a selected group of patients with metastatic disease, confined to lymph node metastases in the cervical or celiac area, who were subjected to resection after achievement of a major response to chemotherapy.

METHODS

Eligibility was restricted to patients with histologically proven squamous cell carcinoma (SCC), adenocarcinoma or non small-cell undifferentiated carcinoma of the thoracic esophagus; pathologically proven measurable metastatic disease at presentation, limited to the cervical or celiac region, as demonstrated by physical examination, chest radiography, ultrasonography of the cervical and upper abdominal region, upper gastrointestinal endoscopy with biopsies, and computed tomography of the chest and abdomen, and bronchoscopy; both a complete regression of the lymph node metastases, and a complete or partial regression of the primary tumor without any sign of new tumor lesions after chemotherapy, as evaluated by upper abdominal endoscopy, computed tomography of the chest,

and ultrasonography of the cervical and celiac regions; age below 75 years; a Karnofsky performance score (P.S.) of 70 or higher; life expectancy greater than 6 months, adequate pulmonary function; adequate bone marrow function after chemotherapy; informed consent. Patients had to be fit enough to undergo an extensive surgical procedure.

Surgery was carried out within 6 weeks after the last chemotherapy course. After laparotomy the abdominal cavity was inspected for metastases, with special attention to the liver and the celiac region. Resection was discarded if metastases could be proven histologically. Thereafter the resectability of the primary tumor was considered. A subtotal esophagectomy was performed, preferably by transhiatal esophagectomy without thoracotomy. The continuity of the digestive tract was restored by a stomach tube reconstruction or colonic interposition procedure with anastomosis in the neck. Patients were followed until death at three months intervals. At each visit during follow up a physical examination and liver biochemistry was performed. Ultrasonography of the cervical and upper abdominal regions and esophagoscopy were performed at 6-months intervals. Other diagnostic tests were performed only when indicated.

The complete absence of any evidence of malignant disease including negative biopsies from the former tumor area was defined as Complete Response (CR). A more than 50% reduction in tumor bulk, but residual disease still evident, was defined as Partial Response (PR). Stable Disease (SD) was defined as neither evidence of progression, nor clear (>50%) reduction of tumor bulk, without the appearance of new lesions. Evidence of progression (>25%) of tumor bulk, progresssion of any lesion, or the appearance of new tumor lesions was defined as Progressive Disease (PD). The tumor stage after resection was defined according to the TNM classification of the International Union Against Cancer (UICC).[2] If no residual tumor was detected, the disease was classified as a pathological complete response. To

describe the absence or presence of residual tumor after resection of the primary, the following R(esidual) categories were used as appendices: R_x if presence of residual tumor could not be assessed, R_0 if all the surgical margins were free of tumor, R_1 if there was microscopic residual tumor in any of the surgical margins, and R_2 if macroscopic residual tumor was detected.

The World Health Organization (WHO) recommendations for grading of toxic effects of chemotherapy were followed.(3)

Survival was measured from the date of diagnosis to the date of death or most recent follow-up visit. Time to distant metastases and time to local recurrence after radical (R0) resection was measured from the date of operation to the occurrence of either event or most recent follow-up visit. Estimates of survival and time to disease recurrence were based on the Kaplan-Meier method.(4)

RESULTS

From December 1990 to July 1996 14 patients were registered. The total duration of follow up after surgery at the time of this writing is 3.3 years, the median length of follow up after surgery for all patients 17 months (range, 3 to 46) . In all patients, evidence of lymph node involvement could be achieved by ultrasound-guided biopsy without the need for explorative laparotomy. One patient was treated with esophageal intubation before chemotherapy. The main patient characteristics are listed in table 1.

Chemotherapy

The median number of chemotherapy cycles was 5 (range, 4 - 6), resulting in a median time from diagnosis to operation of 7.3 months (range, 5.1 to 10.7). Three chemotherapy regimens were used, all as part of prospective phase 2 study protocols

in operation at that time; 3 patients were treated with the combination of cisplatin and etoposide (regimen A)[(5)], one patient with 5-fluoro-uracil and folinic acid (regimen B)[(6)] and 10 patients with the combination of cisplatin, 5-fluoro-uracil, etoposide and folinic acid (regimen C, report in preparation). Details of these regimens are listed in table 2.

There were no drug related deaths. All three chemotherapy regimens were well tolerated. Only three of 71 cycles (4%) had to be postponed 1 week because of treatment related toxicity. Dose reductions of etoposide (2 cycles) and 5-fluoro-uracil (5 cycles) were applied for hematologic and mucosal toxicity reasons. Alopecia was common. There were no signs of cardiac toxicity or hand-foot syndrome. An almost 10 percent increase in median body weight was documented during chemotherapy: from 64.7 kg (range, 51.1 to 81.0) at the start of treatment, to 70.4 kg (range, 52.4 to 85.5) after the fourth cycle.

Eleven of 14 patients (79%) noticed relief of dysphagia during chemotherapy, one patient had no relief, and two had no dysphagia at all at the time of diagnosis. At endoscopy after chemotherapy, a complete regression with negative biopsies was seen in 3 cases; in 3 other patients endoscopy was normal, but biopsies from the former tumor area were still positive.

Surgery

In 2 patients no resection was performed because of liver involvement and tumor infiltration of the right bronchial tree, respectively. In 11 patients a transhiatal resection was done, whereas in one patient a combined thoraco-abdominal approach had to be chosen because of an injury of the thoracic aorta. In one patient a colonic interposition was performed because of stomach surgery in the past; in the other 11 patients a stomach tube reconstruction was used. Fibrosis around the tumor hampered a prompt resection in 3 cases. In 2 patients postoperative hemorrhage required a second surgical

procedure (thoracotomy) within 2 weeks after resection. Other postoperative complications were anastomotic leakage (n=1), laryngeal nerve paralysis (n=1), and pulmonary problems (n=3). Patients were discharged after a median time of 20 days after resection (range, 11 to 37). Postoperative 30-day mortality was nil.

Pathology

Pathology findings at surgery are listed in table 3.
Although a pathological examination of the resected specimen after a transhiatal approach does not permit a reliable determination of the pN category, we did classify the post-chemotherapy residual tumor stage according to the UICC stage classification, on the basis of surgical and pathological data. Chemotherapy effects like necrotic fields, calcifications and diffuse fibrosis were manifest in 6 of 12 specimens (1 adenocarcinoma, 5 squamous cell carcinomas).

Follow-up and Survival

Individual data of follow-up and survival after chemotherapy and surgery are listed in table 4. One patient (pat.no. 10) was treated with radiotherapy (cervical area) at once after resection. In case of local recurrence or distant metastases during follow-up, patients were treated with radiotherapy (pat.no. 1,2,10,12,14), experimental chemotherapy (pat.no. 13) and/or best supportive care.

Median survival time after diagnosis (n=14) was 32 months, after resection (n=12) 26 months. Three year survival (± S.E.) after resection was 27 (± 16) percent. Median time to progression after resection (n=12) was 9 months.

DISCUSSION

This report describes 14 patients with esophageal cancer, with metastatic disease at presentation, limited to lymph node involvement in the cervical and/or celiac region, who were subjected to salvage surgery after chemotherapy. Regarding the diverging eligibility criteria in the subsequent phase II chemotherapy protocols in operation at that time, and the fact that all patients achieved a major response on chemotherapy, the data represent a selected group of patients.

Fibrosis around the tumor in 3 patients was a factor contributing to peri- and postoperative morbidity. Ten of 12 patients who underwent resection, had tumor-free margins. The majority of patients (n=9) was confronted with distant relapses during follow-up. In 2 patients these relapses were located in the same area as at presentation. Although no formal quality of life assessments were made, it is our impression that the quality of life during long term follow-up of this group of patients was similar to that of patients treated with surgery alone at our institution.

There is no standard therapy for metastatic esophageal cancer. Intubation, radiotherapy, endoscopic laser therapy, or combinations of these modalities are the most frequently used palliative treatment options. Most patients die within 3 to 6 months.(7)·(8)·(9)·(10) Combination chemotherapy can result in response rates between 35 and 55 percent, with a median response duration of 4 to 7 months, and a median survival time of 6 to 9 months.(11)

After consolidation radiotherapy in selected patients, who do respond on chemotherapy, the median time to progression can be as high as 11 months.[5] However, even after additional radiotherapy, the primary tumor is often among the first sites of recurrence, leading to dysphagia, weight loss, malnutrition, and a burden to the patients mental and emotional status. Also adequate radiotherapy on the esophagus after prolonged chemo-

therapy carries a risk of developing severe mucositis and -fistulas.

In the current series, only three patients developed a local recurrence as the first site of progressive disease at 13.1, 21.8 and 8.5 months after resection. The median survival time after resection (32 months) compares not badly with that of responding patients with operable disease (44 months, see Chapter 6 of this thesis), considering the different disease stages.

In conclusion, salvage esophagectomy should be considered in patients with esophageal cancer with metastastic disease, confined to the cervical and/or celiac area, who respond well to chemotherapy.

Table 1. Characteristics of patients (n=14)

Pat. ID #	Age (yr)	Sex	P.S. (%)	Weight loss (%)	Hist. type	Hist. grade	Location: up/mid/low	Size (cm)	Location lymph node metastases
1	53	m	90	6	SCC	3	mid	8	cervical (R) + celiac
2	54	m	90	8	SCC	3	mid	10	cervical (R)
3	67	m	80	unknown	SCC	3	up	6	cervical (R) + celiac
4	68	m	90	13	adeno	3	mid	9	cervical (R)
5	70	m	90	0	adeno	3	low	5	celiac
6	71	m	90	unknown	adeno	2	low	5	cervical (L)
7	61	f	90	15	adeno	2	low	7	celiac
8	63	m	80	19	SCC	3	low	4	celiac
9	60	m	90	4	SCC	3	low	8	celiac
10	52	m	90	0	SCC	2	mid	4	cervical (L)
11	54	f	80	3	SCC	3	mid	2	cervical (R)
12	45	m	80	11	SCC	2	low	5	celiac
13	53	m	90	7	SCC	2	mid	5	cervical (R)
14	49	f	90	9	SCC	2	mid	5	cervical (L)

Table 2. Chemotherapy.

Regimen [ref]		Interval	Patients
A[5]	Cisplatin 80 mg/m^2 day 1 iv Etoposide 100 mg iv day 1,2 Etoposide 200 mg/m^2 orally day 3,5	4 weeks	3
B[6]	5-Fluoro-uracil 500 mg/m^2/day x 5 Folinic acid 6 x 60 mg/day x 5	4 weeks	1
C	Cisplatin 80 mg/m^2 day 1 iv Etoposide 125 mg/m^2 iv day 1 Etoposide 200 mg/m^2 orally day 3,5 5-Fluo-uracil 375 mg/m^2/day x 5 Folinic acid 6 x 60 mg/day x 5	4 weeks	10

Table 3. Pathology after resection (n=12)

UICC Stage	
Complete Response	1
I	2
IIA	4
III	2
IV	3
Primary Tumor	
pT0	1
pT1	2
pT2	3
pT3	6
Distant Metastases	3
Cervical lymph node	1
Celiac lymph node	2
Residual Disease	
R0	10
R1	2

Table 4. Follow-up and Survival after Resection (n=14)

Pat. ID #	UICC stage	Time (mo) after res. to loc.recur. / dist.metast.	Location metast.	D(ead)/ A(live)	Survival (mo) after surgery / diagnosis
1	no res. (T4)	0.0 / (unknown)	(unknown)	D	4.9 / 10.4
2	III	13.1 / 13.1	LYM cerv.	D	25.4 / 31.8
3	IIA	- / 2.6	Lung, Liver	D	3.3 / 12.4
4	IV	- / 8.9	LYM cel.	D	9.7 / 19.6
5	IIA	- / -	-	A	46.3 / 53.0
6	IV	- / 2.4	LYM cel.	D	5.9 / 16.6
7	IIA	- / -	-	A	16.9 / 24.8
8	III	- / 5.2	Liver	D	7.9 / 15.5
9	Complete Response	- / -	-	A	38.3 / 46.2
10	I	- / 8.4	LYM cerv.	D	26.0 / 33.5
11	no res. (M1)	0.0 / 0.0	LYM cel.	D	4.7 / 9.8
12	IIA	21.8 / (unknown)	unknown)	D	33.4 / 39.3
13	IV	8.5 / 8.5	LYM cerv.	A	10.1 / 16.1
14	I	- / 11.2	LYM cerv.	A	16.8 / 24.1

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CHAPTER 8

NO EVIDENCE OF KNOWN TYPES OF HUMAN PAPILLOMAVIRUS IN SQUAMOUS CELL CANCER OF THE ESOPHAGUS

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SUMMARY

Controversial results regarding the presence and role of human papillomavirus in the development of esophageal squamous cell carcinoma have been published. We used multiple broad spectrum polymerase chain reactions to identify HPV DNA in esophageal carcinomas from a low incidence area.
Paraffin embedded- and snap frozen specimens from esophageal cancer tissues of 63 patients were examined with a PCR technique with several primer pairs, capable of detecting most known HPV types.
In none of the esophagus cancer tissues could HPV DNA be detected.
The role of HPV in this type of carcinoma in a low incidence area remains unclear.

INTRODUCTION

Squamous cell carcinoma of the esophagus is a highly lethal disease, with a striking variation in incidence in different parts of the world. From epidemiologic surveys, it has been suggested that excessive alcohol intake and use of tobacco (especially in combination), and possibly certain nutritional deficiencies (vitamins A, B, and C) are some of the risk factors, but these factors alone can not explain the very high incidence in some well defined geographical areas in North China, Iran and South Africa.

Human papillomaviruses (HPV) have been found to play a causative role in the pathogenesis of cervical dysplasia and cervical carcinomas. In 1978 a possible interaction between a bovine papillomavirus (BP4) and an environmental carcinogen (bracken fern) has been regarded as an important event for the development of squamous cell carcinomas, especially of the esophagus, in cattle grazing on the Scottish Highlands (1).

Syrjänen suggested in 1982 for the first time a possible etiologic relation between HPV and benign proliferations of the squamous mucous membrane of the esophagus, for instance papillomas (2). In the animal model of bovine papillomavirus infection, these lesions have been reported to undergo malignant transformation following exposure to carcinogens. Winkler et al reported in 1985 about the clinical, histological, and morphological features of HPV infection in cases of benign esophageal proliferations, while at the same time a role of HPV infection in carcinoma of the esophagus in black South Africans was suggested (3,4). In both studies, HPV antigens could be detected by means of immunoperoxidase techniques in 30% of those cases, in which the histologic criteria of HPV infection were met. Since then, a number of controversial studies have been published about the detection of HPV DNA in human esophageal cancer with different techniques. Positive results were obtained mainly in high incidence areas. In the Netherlands, squamous cell cancer of the esophagus is a rare disease. We investigated the presence of HPV DNA in esophageal cancer from a low incidence area using multiple very sensitive broad spectrum polymerase chain reaction techniques.

MATERIALS AND METHODS

We investigated formalin fixed, paraffin embedded tumour specimens of 63 consecutive patients with operable invasive squamous or undifferentiated large cell carcinoma of the esophagus, who participated in a phase III randomized clinical trial of surgery with (n=21) or without (n=42) neoadjuvant chemotherapy. This study was carried out in the largest referral centre for esophageal cancer patients in the Netherlands (age-adjusted death rate of esophageal cancer: $7.9/10^5$ for males and $3.2/10^5$ for females). In 20 out of 42 patients, treated with surgery alone, we also investigated snap-frozen

specimens, collected and frozen in liquid nitrogen within 1 hour after surgical removal. All available hematoxylin eosine stained sections were reviewed, and the most representative block was selected for further studies. Patient characteristics are listed in table 1. No patient had a history of, or a presence of active papillomas of any site in the head and neck or esophagus region.

To extract DNA from paraffin embedded tissue 5 μm sections were cut taking care to prevent cross-contamination and incubated in 300 μl 10mM-Tris.HCl (pH 8.9), 50 mM-KCl, 2.5 mM-$MgCl_2$ and 0.5% Tween-80 with 200 μg/ml proteinase K at 56 °C for 18 hours. At the end of the incubation the aqueous phase was separated from the paraffin slurry by centrifugation, transferred to a fresh tube and the proteinase K was subsequently inactivated by boiling. Frozen tissue specimens were sliced using a cryomicrotome and two 5 μm sections were used for the preparation of DNA. To extract DNA the slices were incubated for 4 hours at 56 °C with 200 μg/ml proteinase K in 200 μl 50 mM-Tris.HCl (pH 8.9) and 1 mM-EDTA. After completion of the digestion the proteinase K was heat-inactivated.

For the analysis of the presence of HPV DNA, 5 μl of each sample was subjected to 40 cycles of PCR amplification in 50 μl reactions using each of the following primer sets: 1. MY09 (5'CGT CC(A/C) A(A/G)A GGG A(A/T)A CTG ATC) and MY11 (5'GC(A/-C) CAG GG(A/T) CAT AA(C/T) AAT GG) (5); 2. GP5 (5'TTT GTT ACT GTG CTA GAT AC) and GP6 (5'TGA TTT ACA GTT TAT TTT TC) (6); 3. CPI (5'TTA TCA (T/A)AT GCC CA(T/C) TGT ACC AT) and CPIIG (5'ATG TTA AT(A/T) (G/C)AG CC(A/T) CCA AAA TT) (7,8) ; 4. CPI and CPIIS (5'ATA TTG TCT GAG CCT CC(A/T) AA(A/G) TT) (9) and 5. Pu1m (5'TGT CAA AAA CCG TTG TGT CC) and Pu2r (5'GAG CTG TCG GCT TAA TTG CTC) (10). Amplification was also done using a nested PCR approach using the MY09/11 primer set in the first and the GP5/6 primer set in the second PCR. Each of these primer pairs was designed for the amplification of spectra of genital HPV types with the exception of the CPI/IIS primers

set, which was developed for the detection of HPV types present in the skin. The MY09/11, GP5/6, Pu1m/2r and CPI/IIG PCRs were performed under the conditions described in the original publications including the appropriate positive (SiHa DNA) and negative controls (5-7,9,10). In addition we used a nested PCR method directed by two novel primer pairs specifically designed for the sensitive amplification of HPV types present in the skin lesions of epidermodysplasia veruciformis patients (11). The nucleotide sequence of the two primers used in the first PCR are 5'CA(A/G) GGT CA(C/T) AA(C/T) AAT GG(C/T) AT (CP65) and 5'AA(C/T) TTT CGT CC(C/T) A(A/G)A G(A/G)A (A/T)AT (CP70), and those of the two primers used in the nested PCR are 5'AAT CA(A/G) (A/C)TG TTT (A/G)TT AC(A/T) GT (CP66) and 5'G(A/T)T AGA TC(A/T) ACA T(C/T)C CA(A/G) AA (CP69).

In a separate reaction a ß-globin PCR was carried out using two pairs of primers; either PC03 (glo-1) and RS42 amplifying a 441 bp fragment or glo-1 (5'ACACAACTGTGTTCACTACC) and glo-3 (5'TCTATTGGTCTCCTTAAACC) amplifying a 172 bp fragment. Successful amplification of the ß-globin fragment, visualized on an ethidium-stained agarose gel, indicated that the sample is adequate for PCR analysis.

RESULTS.

None of the different PCR reactions resulted in the detection of HPV DNA in the esophageal carcinoma specimens when the PCR products were analyzed by ethidium bromide stained agarose gels (figure 1). Also no specific signals were detected after blotting and hybridization of the PCR products of the MY09/11 PCR, the GP5/6 PCR and the CPI/IIG PCR with mixtures of PCR labelled probes. These primer pairs combined are capable of detecting most known HPV types. Seventy eight and 79 of a total of 83 samples were positive when analysed by PCR with the two ß-globin primer pairs producing a 441 and 171 bp

fragment, respectively (figure 1). This indicates that almost all DNA preparations were adequate for PCR analysis.

DISCUSSION

We investigated a possible role of HPV in the pathogenesis of squamous cell carcinoma of the esophagus in patients from a low incidence area. In this material, of which 95% of the specimens were proven to be suitable for PCR analysis, we could not detect any HPV DNA. These results strongly suggest that HPV DNA of the known HPV types is not present in at least the majority of the esophageal squamous cell carcinomas in our country. We can not exclude the possibility that novel HPV types, which do not match the PCR primers used, are present. Recently we succeeded in detecting novel HPV types in skin lesions of immunocompromised patients by PCR amplification, employing a nested PCR approach using the primer sets CP65/66 and CP69/70 (11).

Data from the literature on HPV, related to benign and malignant esophageal lesions, are conflicting (12). In a study from Australia, with DNA hybridization techniques, using a mixed probe of HPV types 11,13,16, and 18, an overt positive reaction in 2, and a weak positive reaction in 3 out of 10 cases of esophageal squamous cell carcinomas could be demonstrated, whereas no detectable viral antigen in these cases was found, suggesting that detection of the genome is more sensitive than immunostaining (13). In 1990 the same author published his results on the positive detection of HPV type 6, 11, 16 and 18 DNA by filter in situ hybridisation (FISH) techniques in 9 out of 39 esophageal cancers from a low incidence area (14). Chang et al reported in 1990 positive results with the same method in esophageal cytologic specimens from a high risk area, ranging from atypia to invasive squamous cell carcinoma, including lesions adjacent to the tumour area (15). In 75% of

positive cases, HPV type 16 and/or 18 DNA sequences were found. These findings were confirmed in several other reports, especially from high risk areas, such as South Africa, Japan, China and France (16-18). The largest series until now was published in 1993 by Chang et al (19). In this study, biopsy specimens of tumor, adjacent epithelia, regional lymph nodes, and surgically resected margins were collected from 363 patients, surgically treated with eso-phagectomy for invasive squamous cell carcinoma in the high risk area of Linxian, China. In 20% of the cases, positive signals were found in the nuclei of cancer cells by in situ hybridization, in 4% only in the adjacent epithelium with hyperplastic or dysplastic changes, and in only 1 case in the resected margin. HPV type 16 was the most common finding in the 85 positive cases (20). The presence of positive hybridization signals in regional lymph node metastases (12.3%), exclusively confined to the nuclei of metastatic cancer cells, suggests a causal association of HPV and esophageal carcinoma in these patients, the moreso because the same viral type was invariably detected in both the primary tumor and the metastatic lesions. Cooper and coworkers demonstrated the presence of HPV DNA in 25 of 48 esophageal cancers from a high risk area (South Africa), utilizing non-isotopic in situ hybridization with HPV DNA probes to HPV 6,11,16,18,31, and 33 (21). Very recently Dillner et al found an association between seropositivity to HPV type 16 and the risk of esophageal cancer in Finland (22).

In contrast to these data, negative results have also been reported, especially in low risk areas, but also in high risk areas (23-26). Loke et al applied both in situ hybridization and DNA slot blot analysis to a series of 37 cases where total esophagectomy was performed for squamous cell carcinoma in the high incidence area of Hongkong. With both techniques no HPV was detectable in cancer cells nor in intra-epithelial neoplasia nor in normal esophageal mucosa. Kiyabu et al used in vitro gene amplification by the polymerase chain reaction to

look for HPV type 16 and 18 DNA in invasive squamous cell cancers of various types (23). While 70% of the ano-genital carcinomas, and 36% of the oro-pharyngeal carcinomas contained HPV DNA sequences, none of 13 esophageal carcinomas was found positive.
Our own results from a low incidence area are consistent with the last mentioned studies.

There are several complicating issues when comparing studies about HPV detection in benign and malignant tissues. In high risk areas a possible influence of screening methods regarding the stage of the tumor at the time of diagnosis could exist. It is important to realize that several authors found HPV DNA in the epithelium, adjacent to the carcinoma, more than in the cancer itself (16). This phenomenon is consistent with the earlier findings, that bovine papillomavirus type 4 DNA in high copy number could be readily identified in bovine papillomas, but no viral DNA nor viral antigens could be detected in malignant lesions itself, indicating that viral genomes are not necessary to maintain a malignant state.
A second issue is the methodology. Using amplification by the polymerase chain reaction, it is possible to detect HPV genomes with a large sensitivity, for instance up to less than 10^{-1} copies of viral genome per cell, which is superior compared to the older hybridization techniques and immunostaining techniques (14,15). We used in this study several pairs of general primer sequences, which are conserved among a broad spectrum of HPV types, and which permit the detection of more HPV types in a single sample. Also potentially new HPV types can be identified by these methods.

In conclusion, in our current study with esophageal squamous cell cancer specimens from a low incidence area, we could not confirm the results of some other investigators, identifying HPV DNA in esophageal carcinomas, mainly type 16 and 18. In this respect, our results are identical to those described by Loke, Kiyabu, Sugimachi and Akutsu (22-25).

Table 1. Patient and tumour characteristics (n=63)

Median age		63.5 years (35-77 yrs)
Gender	male	48
	female	15
Race	caucasian	60
	black	2
	chinese	1
Grade of differentiation		
	well	n= 7
	moderately	n=38
	poor	n=17
	undifferentiated	n= 1
Stage (UICC)		
	I	n= 6 (10%)
	II	n=26 (41%)
	III	n=10 (16%)
	IV	n=21 (33%)

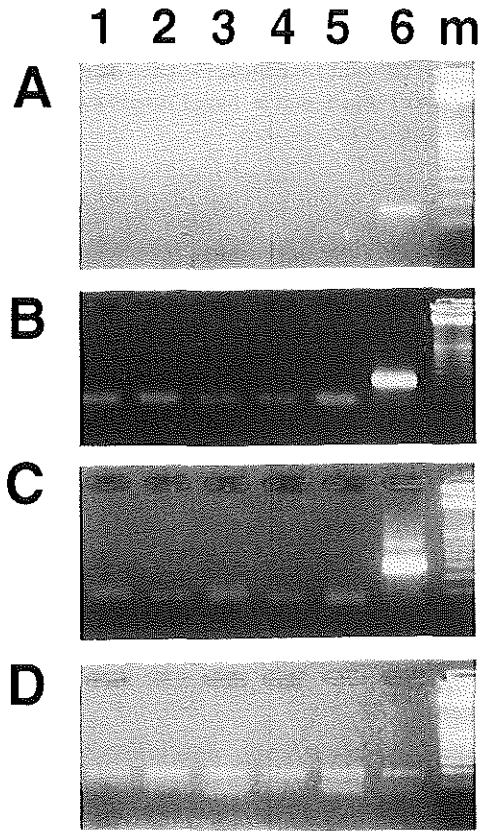

Figure 1:
Absence of HPV DNA from esophageal carcinomas:
Ten μl of the PCR products of 5 tumors (lane 1-5) and SiHa cells containing 1-2 copies of HPV-16 DNA per cell (lane 6) were run on a ethidium bromide stained agarose gel.
Lane m: 100-bp ladder marker. The band of the positive control (Lane 6) indicates the position of expected HPV-amplimers.
Amplimers of the PCR directed by (A) GP5/6 primers, (B) CPI/I-IG primers, (C) Pu1m/2r primers, (D) Glo-1/Glo-3 primers.

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CHAPTER 9

EXPRESSION OF THE MULTIDRUG RESISTANCE PROTEIN (MRP) IN SQUAMOUS CELL CANCER OF THE ESOPHAGUS AND RESPONSE TO PREOPERATIVE CHEMOTHERAPY

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ABSTRACT

One of the major problems in the treatment of squamous cell carcinoma of the oesophagus (ESCC) is the unresponsiveness to cytotoxic drugs. So far, the mechanisms underlying the intrinsic drug resistance of ESCC remain unclear. We determined the expression of a newly recognised drug resistance protein, the Multidrug Resistance Protein (MRP) in tumour biopsies from ESCCs by RNase protection assay (RPA) as well as by immunohistochemistry (IHC) on paraffin-embedded and cryostat tissue sections. The ESCC samples were obtained from patients participating in a prospective randomized clinical phase III trial, evaluating preoperative chemotherapy (cisplatin and etoposide) followed by surgery versus surgery alone in patients with operable ESCC. For most patients included in the study, tumour biopsies taken at diagnosis by endoscopy as well as surgically resected primary tumours were available. Of 58 ESCC patients enrolled 28 received chemotherapy before surgical resection of their tumours, and 30 were treated with surgery alone. Twelve patients (43%) showed a major response after chemotherapy, either be a complete (CR) (11%) or a partial response (PR) (32%), 10 patients (36%) were classified as stable disease (SD), and 6 patients (21%) had progressive disease (PD). IHC was performed with two MRP-specific monoclonal antibodies (MAb). The rat MAb MRPr1 was applied on paraffin-embedded tissue sections, while the mouse MAb MRPm6 was used on cryostat sections. The immunostaining was graded on a semiquantitative scale that ranged from (-) to (+++). When applied on 14 surgically resected, untreated primary ESCCs, the IHC scores correlated positively rather well (Spearman's rank correlation coefficient, r_s=0.8; $p<0.01$), as determined with the two different MAbs, and with the MRP mRNA levels, quantitated by RPA (multiple testing, $p<0.01$). Using MRPr1, MRP expression was detected in the vast majority of the diagnostic biopsies. Overall, 90% of the tumours were posi-

tive for the anti-MRP antibody: 33% showed weak cytoplasmic staining of the tumour cells (±), 33% had a clear cytoplasmic staining (+), in 21% a strong cytoplasmic and weak membranous staining (++) of tumour cells was observed, and in only 3% the tumours cells had strong cytoplasmic as well as strong membranous staining (+++). MRP expression in the primary ESCCs at the time of diagnosis did not correlate with the outcome of subsequent preoperative chemotherapy. For all IHC staining groups (-, ±, +, or ++/+++) the major response rate was about equal, and MRP expression did not differ significantly between CR and PR, and patients with SD or PD. In addition, multivariate analysis by logistic regression did not show any effect of tumour cell differentiation or UICC tumour stage on the outcome of preoperative chemotherapy in relation to MRP expression. However, a difference became apparent (Sign-test, $p=<0.05$) for higher MRP expression in tumours from patients with PR or SD, when comparing MRP levels in paired tumour samples before and after chemotherapy, suggesting that chemotherapy selected for drug-resistant cell clones. In conclusion: i) MRP expression is widely found in primary ESCCs; ii) MRP expression at diagnosis does not appear to be correlated with response on preoperative chemotherapy; and iii) Higher MRP expression is found in tumours from patients with PR or SD, probably due to selection of drug-resistant cell clones. Further studies are needed to elucidate the mechanisms of clinical drug resistance in ESCC.

INTRODUCTION

Standard treatment for squamous cell carcinoma of the oesophagus (ESCC) consists of surgical resection. Cure is rare, with 5-year survival of less than 10%. Response rates to chemotherapeutic agents, given singly or in combination, are low and range from 15-40% [1]. However, a promising develop-

ment in the treatment of ESCC is the use of preoperative chemotherapy with the primary goal of debulking the tumour load, followed by surgical resection of the tumour. In 1989, a multicentre prospective randomized phase III trial was initiated in the University Hospital Rotterdam (The Netherlands), in which preoperative chemotherapy (cisplatin and etoposide) followed by surgery was compared with surgery alone in patients with operable ESCC. Clinical response to chemotherapy was evaluated after the second cycle, and patients with a major response were given two additional cycles of chemotherapy followed by surgery, whereas non-responding patients were operated at once. Interim analysis showed very favourable results in terms of survival for those who responded well to preoperative chemotherapy [2]. The median survival after randomization was 18.5 months for the "chemotherapy plus surgery" arm, versus 11 months for the "surgery alone" arm ($p=0.0017$). Among the chemotherapy-treated patients, about 40% had a major response (with about 10% complete responders).

In a previous study [3] we have shown that a variety of human cancers, including ESCC, overexpress the newly recognized drug resistance gene, the Multidrug Resistance Protein (MRP) gene [4]. MRP encodes a 190 kDa membrane bound glycoprotein of 1531 amino acids and is a member of the ATP-binding cassette superfamily of transport proteins [4, 5]. Transfection experiments with different eukaryotic expression vectors containing full-length complementary DNAs of the MRP gene have shown that MRP confers multidrug resistance (MDR) to a broad range of natural product drugs, among which are anthracyclines, vinca alkaloids and epipodophyllotoxins [6-9]. As yet, the mode of action by which MRP makes cells MDR is not known. However, the available data suggest that MRP acts both as a plasma membrane outward drug pump and as a pump for drug accumulation in intracytoplasmic vesicles [6, 7, 10, 11]. By both mechanisms cytoplasmic concentrations of free drug may be reduced to sublethal levels, and in that way MRP would promote

cell survival. Although, expression of MRP has been demonstrated in a variety of solid tumours [3, 12-20], and leukaemias [21-23], the question whether elevated levels of MRP are associated with clinical drug resistance has not been fully answered, yet. In the present study we have examined the hypothesis that unresponsiveness of ESCC to chemotherapy might be related to the presence of MRP.

MATERIALS AND METHODS

Tumour samples

Tissue samples were subjected to this research with informed consent of the patients. In total, 58 primary ESCCs, resected at the University Hospital Rotterdam between 1989 and 1993 were analysed for MRP expression. The ESCC samples were obtained consecutively in a prospective randomized clinical phase III trial, evaluating preoperative chemotherapy (cisplatin and etoposide) followed by surgery versus surgery alone in patients with operable ESCC. All chemotherapy-treated patients received 2 cycles of chemotherapy. Patients with a major response, as defined by a tumour reduction of >50%, were given 2 additional cycles of chemotherapy, followed by surgery, whereas patients with stable (SD) or progressive disease (PD), were subjected to surgery at once. Cisplatin (80 mg/m^2) was given at day 1 as a 4-h infusion preceded by etoposide (100 mg) as a 2-h infusion. On day 2 only etoposide (100 mg) was given as a 2-h infusion, and on day 3 and 5 etoposide (200 mg/m^2) was administered orally. The specimens included in the study were 30 tumours resected without prior chemotherapy, and 28 tumours for which patients received chemotherapy before surgical resection of their tumours. Clinical response to preoperative chemotherapy was categorized as complete (CR), indicating the pathologic absence of tumour in the resection; or partial (PR), indicating at least a 50% reduction in the

size of the tumour. SD was defined as no change in tumour volume, and in the case of PD tumour volume increased during treatment. The reduction in tumour size was estimated by comparing tumour dimensions obtained before and after chemotherapy by endoscopic and radiologic examination. Endoscopic biopsies and resected tumours were routinely processed at the pathology department of the hospital for diagnostic purposes. Patient and tumour characteristics are summarized in Table 1.

RNase protection assay

Total RNA was isolated from tissue biopsies by the lithium-chloride-urea method [25], and quantitated spectrophotometrically at A260. Expression of MRP mRNA was quantitated by RNase protection assay (RPA) as described previously [21, 26]. Briefly, 10 μg of total RNA were hybridised under standard conditions with α-^{32}P labeled RNA transcripts complementary to sequences (nucleotides 239-503) at the 5' end of the MRP mRNA [4, 26]. This probe does not cross-react with the human MDR1 or MDR3 mRNAs [21]. Radiolabeled protected probes were visualised by electrophoresis through a denaturing 6% acrylamide gel, followed by autoradiography. In all assays a human γ-actin probe was included as control for RNA integrity and recovery. All individual experiments included tRNA, as well as RNA isolated from the drug resistant cell line GLC4/ADR and its parental cell line GLC4 (kindly provided by Dr. E. G. E. de Vries, University of Groningen, Groningen, The Netherlands), as positive and negative controls [26]. Expression levels were quantitated by densitometric scanning of the autoradiographs.

Immunohistochemistry

MRP expression was estimated by immunohistochemistry (IHC) on paraffin-embedded tissue sections (5 μm) prepared from tissue blocks used for routine diagnostic purposes, as well as on cryostat sections (5 μm) prepared from frozen biopsies. Sec-

tions of paraffin-embedded tissue were placed on slides coated with 3-aminopropyl-triethoxysilane (Sigma) in aceton (1:50), dried overnight at 37 °C, deparafinized, rehydrated, and washed in phosphate-buffered saline (PBS: 0.13 mol/L NaCl, 7 mmol/L Na2HPO4, 3 mmol/L NaH2PO4, pH 7.6). Cryostat sections were fixed in cold aceton (10 min, 0 °C) and air-dried. Endogenous peroxidase activity was blocked by emerging the slides for 30 min in 0.3% (v/v) H2O2 in methanol at room temperature. Endogenous avidin and biotin activity was blocked by the avidin and biotin blocking kit (Vector Lab). The slides were preincubated with normal goat serum (NGS) (Gibco) (1:50) diluted in 1% (v/v) bovine serum albumin (BSA) in PBS for 20 min at room temperature. Incubation with the MRP-specific monoclonal antibodies (MAb) was performed overnight at 4 °C. Two MAbs specific for MRP were used: the rat MAb MRPr1 and the mouse MAb MRPm6 [27]. Prior to use, MRPr1 and MRPm6 were diluted 1:1500 and 1:25, respectively in PBS containing normal rabbit serum (Gibco) (10%, w/v), NGS (1%, w/v), and normal human AB serum (NHS) (Sigma) (1%, w/v). Subsequently, the slides were incubated for 60 min with biotinilated goat-anti-rat MAb or goat anti-mouse MAb (Sigma) diluted 1:50 in PBS supplemented with 1% BSA, 2% NHS and 2% NGS. Antibody binding was detected using streptavidin-conjugated horseradish peroxidase (Zymed) (1:200 in PBS with 1% BSA) in combination with 3, 3'-diaminobenzidine tertrahydrochloride (Sigma). The slides were counterstained with haematoxylin and mounted. The specificity of MRPr1 and MRPm6 has been documented in detail elsewhere [3, 13, 27, 28] and are suitable for protein blot analysis, flow cytometry, and immunohistochemical studies. MRPr1 and MRPm6 do not cross-react with the human MDR1 and MDR3 glycoproteins [27]. Paraffin-embedded or frozen, MRP-overexpressing, drug-resistant human lung cancer cell line GLC4/ADR and its drug-sensitive parental line GLC4 were used in each assay as positive and negative controls, respectively. Each assay included also the use of isotype-matched irrelevant MAbs (rat IgG2a;

mouse IgG1). Staining of the tumour cells was scored on the following semiquantitative scale: negative with only weak staining of the stromal tissues (-); weak cytoplasmic staining of the tumour cells (±); clear cytoplasmic staining of the tumour cells (+); strong cytoplasmic and weak membranous staining of the tumour cells (++), and strong cytoplasmic and strong membranous staining of the tumour cells (+++). The MRP staining was scored by two independent observers (F.T. B., K. v. W.), one of which is a board-certified pathologist (F.T. B.), who had no further clinical information of the patients whose tumours were analysed.

RESULTS

Expression of MRP protein and mRNA

MRP expression was determined by IHC in 14 surgically resected, primary untreated ESCCs. MRPr1 was used on paraffin-embedded sections, and MRPm6 on cryostat sections. The vast majority of the tumour samples stained with MRPr1 as well as with MRPm6, while incubation with the control, irrelevant rat and mouse MAbs was always negative. The intensity and cellular localisation of the staining varied among the different ESCC samples, and based on these parameters the tumour samples were qualitatively divided into 5 groups (-, ±, +, ++, +++). No staining was denoted as (-), weak cytoplasmic staining of the tumour cells as (±), clear cytoplasmic staining as (+), strong cytoplasmic and weak membranous staining of tumour cells as (++), and (+++) represented strong cytoplasmic as well as strong membranous staining of the tumour cells. As the intensity of the MRP staining increased, the percentage of stained tumour cells increased also. The IHC staining group ±, and + mostly had between 30-50% of the tumour cells stained, while for the stronger stained tumours (IHC score: ++, +++) this figure generally was more than 50%. Since MRPr1 has a higher

affinity than MRPm6 (26), it was used in a higher dilution than MRPm6 (1:1500 versus 1:25). The IHC scores obtained with the two different MAbs matched rather well (Spearman rank correlation coefficient, r_s=0.8; p<0.01) (Table 2).

To correlate MRP protein expression, as estimated by IHC, with mRNA expression, MRP mRNA levels were determined with a sensitive and quantitative RPA in RNA samples isolated from freshly obtained ESCC biopsies, as described previously [3, 21, 22, 26]. The MRP-overexpressing drug-resistant lung cancer cell line GLC4/ADR and its drug sensitive parental cell line GLC4, were used in each experiment as positive and negative controls, and to compare MRP expression levels in different experiments. Expression levels were quantitated by densitometric scanning of the autoradiographs and the signal obtained with 10 μg of total RNA, isolated from GLC4/ADR cells, was assigned an arbitrary expression level of 100 U. In all 14 ESCC samples we could detect MRP mRNA (Table 2). The expression levels among the various tumours ranged from 2 to 33 U. These MRP mRNA levels of the ESCC samples, quantitated by RPA, strongly correlated with the MRP protein levels, estimated by IHC (Spearman rank correlation, multiple testing p<0.01).

MRP expression in endoscopic biopsies

MRP expression was determined by IHC with MRPr1 on paraffin-embedded sections of endoscopic biopsies of 58 primary, untreated ESCCs. Fifty-two of 58 (90%) of the tumour samples stained with MRPr1, while incubation with the control, irrelevant rat MAb was always negative. The intensity and cellular localisation of the staining varied among the different ESCC samples from -, ±, +, ++, to +++ with regard to MRPr1 staining. Six of 58 (10%) ESCC specimen were scored as negative, 19/58 (33%) showed weak cytoplasmic staining of the tumour cells (±), 19/58 (33%) had a clear cytoplasmic staining (+), in 12/58 (21%) the staining was scored as ++, and in only

2/58 (3%) as +++. The intensity of the staining in the highest MRP staining group (IHC score: +++) equals the intensity observed in the MRP-positive, drug-resistant GLC4/ADR cells.

Twenty-eight of 58 patients received preoperative chemotherapy with cisplatin and etoposide, and the clinical response of the primary tumour is shown in Table 3. Twelve patients (43%) reacted with a major response, either be a CR (11%) or a PR (32%), 10 patients (36%) were classified as SD, and 6 patients (21%) had PD. The IHC MRP score of the diagnostic biopsies of the tumours before chemotherapy are also shown in Table 3. No correlation seems to be present between the IHC score and response on chemotherapy. Figure 1 shows the percentage of major response (CR plus PR) of each IHC staining group. Again, no correlation becomes evident between IHC staining and response on chemotherapy (X^2-test, n.s.).

We next stratified the patients on the basis of tumour cell differentiation and tumour stage, and analysed MRP expression in relation to clinical outcome. Tumour cell differentiation was dichotomized into differentiated, taking together well - and moderately differentiated tumours, and undifferentiated, representing poorly - and undifferentiated tumours. In the stratification on tumour stage, the tumours were divided into two categories: locoregional disease (UICC stage I, II, and III) versus stage IV (metastasized tumour). The response on chemotherapy was split into major responders (CR and PR) and non-responders (SD and PD). Multivariate analysis by logistic regression did not show any effect of tumour cell differentiation and UICC tumour stage on the outcome of preoperative chemotherapy in relation to MRP expression.

MRP expression before and after chemotherapy

The resected tumours available for MRP IHC included 30 tumours resected without prior chemotherapy, and 21 tumours for which patients had received chemotherapy before surgical resection. No differences were found in MRP expression between

the treated and untreated tumours (Table 4). However, when MRP expression is compared in paired samples of diagnostic biopsies and resected tumours, obtained after chemotherapy, we see a statistically significant difference for higher MRP expression upon drug treatment in SD and PR (Sign-test, p=<0.05). This phenomenon is not observed in PD, and in the control "surgery alone" group. In SD and PR, respectively 7/9 (78%) and 5/7 (71%) have an increased MRP expression after chemotherapy (Figure 2). For the patients with PD and the "surgery alone" group these figures are 2/5 (40%) and 4/13 (31%), respectively.

DISCUSSION

One of the major problems in the treatment of squamous cell carcinoma of the oesophagus (ESCC) is the unresponsiveness to cytotoxic drugs. So far, the mechanisms underlying the intrinsic drug resistance of ESCC remain unclear. The well-known drug resistance mechanisms that have been elucidated in the laboratory using cell lines made drug-resistant by *in vitro* challenging, are probably not involved in ESCC. Enhanced cellular drug efflux by the P-glycoprotein drug pump [reviewed in: 29], decreased or altered topoisomerase IIα [30], and changes in cellular detoxification systems [31], have not been shown to play a role in the unresponsiveness of ESCC to chemotherapy. In the present study we analysed the expression of the newly recognized drug resistance MRP gene in relation to response on chemotherapy in ESCCs obtained in a preoperative chemotherapy phase III study. MRP expression was determined by IHC with two different MRP-specific MAbs, MRPr1 and MRPm6, directed against two different epitopes of the MRP protein [27]. A good match was found between the IHC scores obtained with the two MAbs. In addition, the protein levels, estimated by IHC, correlated with MRP mRNA levels, as quantitated by

RPA. Subsequently, we used the MRPr1 antibody for detection of MRP in paraffin-embedded sections of diagnostic biopsies. The vast majority of the diagnostic biopsies were positive for MRP, and the protein level, as estimated by IHC, varied between low to very high. Our study aimed at the elucidation of a possible role for MRP in clinical drug resistance in ESCC. In classical adjuvant strategies, the efficacy of chemotherapy cannot be assessed directly, because drug treatment is given after surgery when measurable tumour is not present any longer. However, in the preoperative chemotherapy setting, when cytoreductive drugs are administered before surgery, the effects of therapy can be measured more accurately. Indeed, the present phase III study, in which preoperative chemotherapy (cisplatin and etoposide) followed by surgery was compared with surgery alone in patients with operable ESCC, allowed us to do so; moreover, because the response on preoperative chemotherapy in our study cohort varied dramatically from CR (11%) to PD (21%). No correlation was found between MRP-staining of the tumours at diagnosis, tumour stage and differentiation, and response to subsequent chemotherapy. The intensity of MRP staining was more or less equally distributed over the clinical response groups, being CR, PR, SD, and PD, and all IHC staining groups (-, ±, +, ++, +++) had an about equal response rate. Expression of MRP is widely distributed among a large variety of normal and malignant tissues [3, 12-23, 26-28]. However, evidence suggestive for a role of MRP in clinical drug resistance has only been scarecely delivered [18-20]. A significant association was found between high levels of MRP expression and poor treatment outcome in neuroblastoma [18], independent of N-myc amplification. In two recent studies [19, 20], we showed that in breast cancer MRP might be of prognostic significance. Our data suggest that MRP is related to resistance to chemotherapy in adjuvant setting [19] as well as in advanced disease [20]. In our present study, a tendency became apparent towards higher MRP expres-

sion in tumours from patients with PR or SD, when comparing MRP levels in paired tumour samples before and after chemotherapy. This observation suggests that chemotherapy either directly enhanced the expression of MRP in the tumour cells by *e.g.* transcriptional activation or that chemotherapy selected for cell clones with higher MRP-expression levels, already present in the tumour before treatment. Indeed, for the MDR1-/P-glycoprotein drug pump stress-induced activation of the MDR1 promoter has been reported [32]. In our study selection of drug-resistant cell clones seems to be likely, since the phenomenon of enhanced MRP expression levels after chemotherapy was more prominent in responsive tumours, including SD. Etoposide belongs to the spectrum of drugs involved in the multidrug-resistance phenotype of MRP, and could account for such an *in vivo* selection. MRP has not been shown to confer resistance to cisplatin *in vitro*. However, MRP might function as an efflux pump for glutathione S-conjugates [33-35], and cisplatin can be a substrate for glutathione conjugation [36]. Therefore, theoretically cisplatin might also contribute to such an *in vivo* selection. In conclusion: MRP expression is widely found among ESCCs and does not seem to predict unresponsiveness for chemotherapy. These results indicate that drug resistance in ESCC is due to yet unkown mechanisms, and further studies are needed to elucidate these mechanism of clinical drug resistance in ESCC.

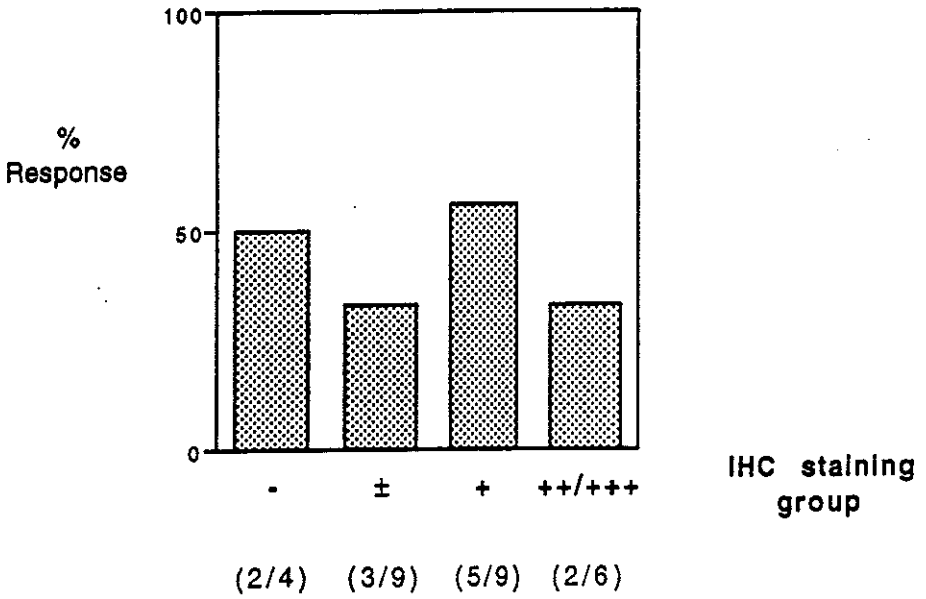

Figure 1
Major clinical response, as defined by reduction in tumour volume of >50%, on preoperative chemotherapy in relation to MRP expression. Between brackets: number of responses per total number of patients in the respective IHC staining groups.

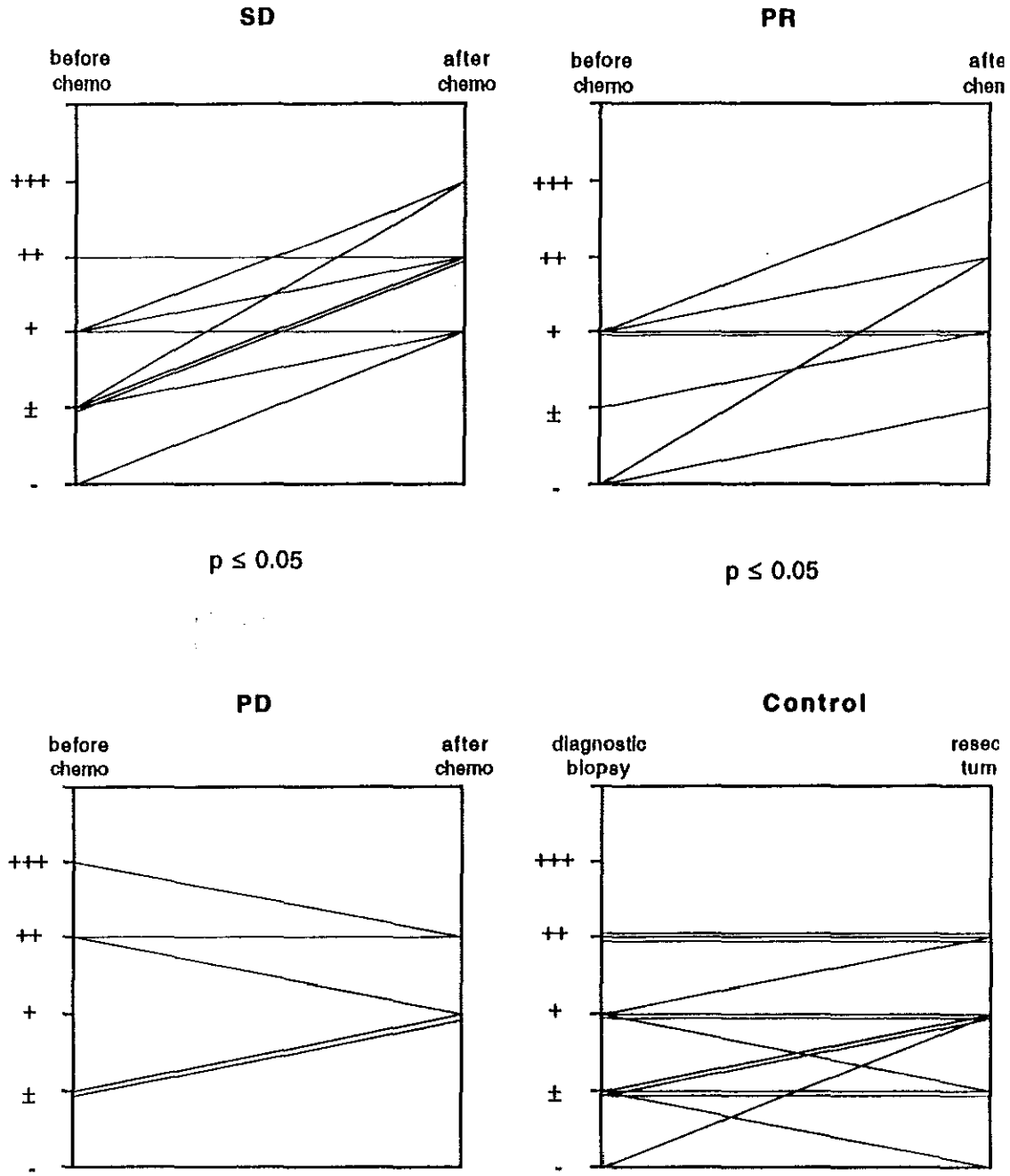

Figure 2

MRP expression in paired tumour samples, before and after chemotherapy, from patients with stable disease (SD), partial response (PR), or progressive disease (PD). In PR and SD expression of MRP was increased significantly (Sign-test, p=<0.05) after chemotherapy.

Table 1.Patient and tumour characteristics of 58 patients with squamous cell carcinoma of the oesophagus

Median age		60.4 years (range 36-77)
Gender	male	43
	female	15
Race	caucasian	58
Grade of tumour differentiation		
	well	n=4 (7%)
	moderately	n=34 (58%)
	poorly	n=19 (33%)
	undifferentiated	n=1 (2%)
Tumour stage (UICC) [a]		
	I	n=7 (12%)
	II	n=22 (38%)
	III	n=6 (10%)
	IV	n=23 (40%)

[a] Ref. 24

Table 2. MRP expression determined by immunohistochemistry and RNase protection assay in surgically resected, primary untreated ESCC

Tumour	Immunohistochemistry [a]		RNase protection assay [b]
	MRPr1	MRPm6	
1	++	++	33
2	+++	+	31
3	++	++	27
4	+	+	17
5	++	++	14
6	++	ND [c]	13
7	++	++	11
8	+	±	9
9	++	+	8
10	+	+	8
11	±	±	7
12	+	ND [c]	5
13	-	-	3
14	±	-	2

[a] Staining of the tumours was scored according to the following categories: negative (-); weak cytoplasmic staining of the tumour cells (±); clear cytoplasmic staining of the tumour cells (+); strong cytoplasmic and weak membranous staining (++), and strong cytoplasmic and strong membranous staining of the tumour cells (+++). MRP-specific MAb MRPr1 was applied on paraffin-embedded tissue sections, while MRPm6 was used on cryostat sections.

[b] MRP mRNA levels were determined with an RNase protection assay [3, 21, 26], and expressed in arbitrary units (U). Each RNA isolate obtained from each individual sample was determined twice, i.e., in two separate assays, and the average of each sample was used to calculate the expression relative to the expression of MRP in the human non-Pgp MDR cell line, GLC4/ADR, which was set arbitrarily at 100 U.

[c] ND, not done, because of lack of frozen material.

Table 3. MRP expression in primary ESCC (n=28) at time of diagnosis versus clinical response on chemotherapy

Clinical response [a]	Immunohistochemical score [b]				
	-	±	+	++	+++
CR (n=3)		1	1	1	
PR (n=9)	2	2	4	1	
SD (n=10)	2	4	3	1	
PD (n=6)		2	1	2	1

[a] Response to preoperative chemotherapy was categorized as complete (CR), indicating the pathologic absence of tumour in the resection; or partial (PR), indicating at least a 50% reduction in the size of the tumour. Stable disease (SD) was defined as no change in tumour volume, and in the case of progressive disease (PD) tumour volume increased during treatment. The degree of reduction in tumour size was estimated by comparing tumour dimensions obtained before and after therapy by endoscopic and radiologic examination.

[b] Staining of the tumours was scored according to the following categories: negative with only weak staining of the stromal tissues (-); weak cytoplasmic staining of the tumour cells (±); clear cytoplasmic staining of the tumour cells (+); strong cytoplasmic and weak membranous staining of the tumour cells (++); strong cytoplasmic and strong membranous staining of the tumour cells (+++).

Table 4. MRP expression of surgically resected squamous cell ca: cinoma of the esophagus estimated by immunohistochemistry with t MRP-specific MAb MRPr1

	Immunohistochemical score				
Patient treatment [a]	-	±	+	++	+++
Untreated (n=30)	2 (6%)	3 (10%)	11 (37%)	11 (37%)	3 (10%)
Treated (n=21)	0 (0%)	4 (19%)	9 (43%)	5 (24%)	3 (14%)
All tumours (n=51)	2 (4%)	7 (14%)	20 (39%)	16 (31%)	6 (12%)

[a] The ESCC samples were obtained in a prospective randomized clinical phase I trial, evaluating the role of preoperative chemotherapy (cisplatin and etoposid followed by surgery versus surgery alone in patients with operable ESCC.

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SUMMARY

CONCLUSIONS AND PERSPECTIVE

Patients with cancer of the esophagus have a dismal outlook. Even after a resection with curative intent, approximately half of the patients die within one year, often with metastatic disease. Although the addition of radiotherapy before or after surgery has led to a somewhat higher resectability rate and a reduction in locoregional failure after resection, no survival advantage has been shown. Obviously, there is a need for effective systemic treatment, alone or in combination with local approaches. In the first part of this thesis (chapters 2 to 5), results of several phase II studies are presented in patients with metastatic squamous cell and adenocarcinoma. The second part (chapters 6 and 7) describes the role of chemotherapy in operable disease, and of salvage surgery in metastatic disease. In the last part (chapters 8 and 9), preclinical experiments are presented regarding aetiology and drug resistance of esophageal cancer.

Chapter 1 presents a synopsis of the aetiology, diagnosis and staging, and local treatment modalities of esophageal cancer. The current knowledge on systemic treatment in this disease is reviewed.

Chapter 2 presents the results of a study with the combination of cisplatin and etoposide in 73 patients with metastatic squamous cell cancer. The overall response rate was 45 percent. Although two toxic deaths occurred, the overall toxicity was manageable.

Chapter 3 describes the toxicity and activity of 5-fluorouracil modulated by folinic acid in 27 patients with metastatic esophageal adenocarcinoma. The response rate was 19 percent, and there were no complete remissions. Toxicity was very mild. This regimen represents suboptimal treatment intensity.

Chapter 4 presents the results of treatment with ifosfamide in 25 patients with advanced adenocarcinoma of the esophagus. Only two patients responded; one however with a continuing complete response of more than 8 years. Single agent ifosfamide has no role in the treatment of adenocarcinoma of the esophagus.

Chapter 5 shows in a small cohort of patients with metastatic esophageal squamous cell carcinoma, that the combination of 13-*cis*-retinoic acid and alpha-interferon was not effective, but this finding should not preclude the investigation of these differentiating compounds in adjuvant treatment settings.

Chapter 6 presents the data of a multicentre prospective randomized trial in patients with operable squamous cell carcinoma. One hundred sixty three patients were randomly assigned to treatment with neoadjuvant chemotherapy (as discussed in chapter 2) followed by surgery, or surgery alone. A clear advantage after preoperative chemotherapy was seen, both in terms of overall survival, and time to local progression and distant metastases. It should be emphasized that this advantage was even more pronounced in responding patients. One patient died of chemotherapy induced toxicity. After chemotherapy the rate of resections with tumor-free margins was significantly higher as compared to the surgery alone group. The chances are that neoadjuvant cisplatin combination chemotherapy represents the most significant contribution to improve the probability of long term survival in esophageal squamous cell carcinoma.

Chapter 7 shows that a salvage esophagectomy should be considered in patients with limited lymph node metastases of esophageal cancer, who respond well to chemotherapy.

Chapter 8 discusses the role of human papillomavirus in esophageal cancer. With PCR techniques, no DNA of any known type of papillomavirus could be detected in 63 resected specimens.

Chapter 9 presents a study aimed to clarify a possible role of the Multidrug Resistance Protein (MRP) in clinical drug resistance in esophageal squamous cell carcinoma. MRP was widely detected and no strong correllation could be found between MRP-staining of the tumors at diagnosis, and response to subsequent chemotherapy. A tendency became apparent towards higher MRP expression in responding tumors, which was explained by selective cell kill.

Perspectives

In metastatic esophageal cancer, single agent chemotherapy has minor activity with response rates of 20 percent or less. The same applies to cell differentiating compounds, although their potency may lie in the treatment of microscopic residual disease. Cisplatin combination chemotherapy seems to have the best hands in metastatic disease. The combination of cisplatin with new compounds like paclitaxel or vinorelbine seems promising. The toxicity profile of these drugs permits intensification programs, which may increase overall response rates and the number of complete responses.

Adequate neoadjuvant combination chemotherapy seems one of the most promising contributions to increase the cure rate in esophageal cancer. In light of the difference in survival advantage between patients who do respond to chemotherapy and those who do not, it seems important to predict the responsiveness of patient and tumor as soon as possible after diagnosis. For this purpose, specific tools have to be developed.

In the prospect of highly active systemic treatment, the present local modalities, i.e. surgery and radiotherapy, will have to demonstrate their value in multimodality approaches once more.

Patienten met een slokdarm-carcinoom hebben een slechte prognose. Ongeveer de helft van hen overlijdt binnen 1 jaar na een radicaal uitgevoerde resectie, vaak ten gevolge van uitzaaiingen van de ziekte. Radiotherapie voor of na de operatie vergroot enigzins de kans op een succesvolle resectie, en een langdurige lokale controle, maar heeft vooralsnog niet geleid tot overlevingswinst. Gezien het systemische karakter van de ziekte, lijkt het gebruik van chemotherapie, eventueel in combinatie met lokale behandelingsmethoden, zinvol.

In het eerste deel van dit proefschrift (hoofdstukken 2-5) worden de resultaten beschreven van een aantal fase 2 studies bij patienten met een gemetastaseerd slokdarmcarcinoom. In het tweede deel (hoofdstuk 6 en 7) wordt de invloed van preoperatieve chemotherapie bij patienten met een operabel carcinoom beschreven, en de resultaten van resectie na chemotherapie bij patienten met een "beperkt" gemetastaseerd carcinoom. Het laatste deel (hoofdstuk 8 en 9) is een verslag van enkele laboratoriumexperimenten, aangaande de etiologie van slokdarmkanker, en de resistentie tegen chemotherapie.

Hoofdstuk 1 betreft een samenvatting van hetgeen bekend is over het ontstaan van slokdarmkanker, de diagnostiek- en stageringsmethodieken, en de resultaten van lokale therapie. Vervolgens wordt de huidige stand van zaken betreffende chemotherapie bij slokdarmkanker weergegeven en besproken.

Hoofdstuk 2 geeft de resultaten weer van een behandeling met cisplatinum en etoposide bij 73 patienten met een gemetastaseerd plaveiselcelcarcinoom van de slokdarm. Vijf en veertig procent van de patienten reageerde met een duidelijke tumorreductie. Twee patienten overleden aan de schadelijke gevolgen van de behandeling. Overigens waren de bijwerkingen in het algemeen acceptabel.

Hoofdstuk 3 bevat de resultaten van een behandeling met 5-fluoro-uracil en folinezuur bij 27 patienten met een gemetastaseerd adenocarcinoom. De bijwerkingen van deze therapie waren mild, maar slechts 19 procent van de patienten vertoonde een partiele remissie. Het gebruikte therapie schema is bij deze patientengroep waarschijnlijk suboptimaal.

Hoofdstuk 4 is een weergave van de behandelingsresultaten met ifosfamide bij 25 patienten met een gemetastasteeerd adenocarcinoom. Alhoewel 1 patient een, nu al meer dan 8 jaar persisterende, complete remissie heeft bereikt, lijkt monotherapie met dit cytostaticum bij deze patientengroep niet effectief.

Hoofdstuk 5 geeft de onderzoeksresultaten weer van een behandeling met de combinatie 13-*cis*-retinoïne zuur en alpha-interferon in een klein patientencohort met een gemetastaseerd plaveiselcelcarcinoom. Alhoewel geen partiele of complete remissies werden gezien, is niet gezegd dat deze combinatie van cel-differentiatie inducerende agentia, niet effectief zou kunnen zijn in een adjuvante setting.

Hoofdstuk 6 is de weerslag van een gerandomiseerde multicenter studie bij 163 patienten met een resectabel plaveiselcel carcinoom. Er werd gerandomiseerd tussen neo-adjuvante chemotherapie (met een schema zoals besproken in hoofdstuk 2) gevolgd door operatie, versus operatie alleen (controle groep). Er werd een duidelijk nut gezien van neo-adjuvante chemotherapie, zowel ten aanzien van de totale overleving, als de ziekte-vrije overleving. Met name die patienten, die gunstig reageren op de chemotherapie, lijken voordeel te putten uit de gecombineerde behandeling. In de met chemotherapie voorbehandelde groep was het aantal radicale resecties duidelijk hoger dan in de controle groep. Een patient overleed aan de schadelijke bijwerkingen van chemotherapie. Hiermee lijkt voor het eerst aangetoond, dat preoperatieve, cisplatinum bevattende,

chemotherapie een significante bijdrage kan leveren aan de verlenging van de overleving van patienten, na operatieve resectie van een plaveiselcelcarcinoom.

Hoofdstuk 7 laat zien dat bij patienten met initieel gemetastaseerde slokdarmkanker, waarvan de metastasen zich beperken tot lymfeklieren in het coeliacus gebied en/of de supraclaviculaire regio, na een gunstige respons op chemotherapie, alsnog operatieve behandeling van de primaire tumor moet worden overwogen.

Hoofdstuk 8 bevat de resultaten van een onderzoek naar de mogelijke rol, die het humaan papillomavirus zou spelen bij slokdarmkanker. In een serie van 63 resectie preparaten konden geen DNA sporen van dit virus worden aangetoond.

Hoofdstuk 9 is een verslag van een onderzoek naar de mogelijke rol van het Multidrug Resistance Protein (MRP) in de resistentie van het plaveiselcelcarcinoom tegen cytostatica. Het MRP kon wijd verspreid worden aangetoond in tumorpreparaten, maar er kon geen duidelijk verband worden gelegd tussen een positieve MRP-kleuring in de tumorbiopten ten tijde van de diagnosestelling, en de respons op chemotherapie. De MRP expressie lijkt wat hoger te zijn in tumoren met een gunstige respons op chemotherapie, hetgeen verklaard kan worden door selectieve cel destructie.

Perspectief

Monotherapie bij patienten met gemetastaseerde slokdarmkanker heeft slechts minimaal resultaat; hoogstens 20% van de patienten bereikt hiermee een remissie. Hetzelfde kan worden gezegd van cel-differentiatie inducerende agentia, alhoewel hun invloed belangrijk zou kunnen zijn in situaties waarbij sprake is van microscopisch tumor residu. Bij gemetastaseerde ziekte lijken de beste resultaten te kunnen worden behaald met cis-

platinum bevattende combinatie-chemotherapie. Zo zijn de eerste resultaten van combinaties met paclitaxel of vinorelbine veelbelovend. Bovendien lijkt de toxiciteit van deze combinaties een intensivering van de behandeling, met het doel het aantal, met name complete, responses te vergroten, niet in de weg te staan.

Effectieve neoadjuvante combinatie chemotherapie lijkt een veelbelovende stap te zijn in de richting van een grotere kans op curatie voor patienten met slokdarm kanker. Gezien het grote verschil in overlevingswinst tussen patienten met een gunstige reactie op chemotherapie ten opzichte van hen die dit niet bereikt hebben, moet gezocht worden naar instrumenten waarmee de individuele chemotherapie gevoeligheid zo snel mogelijk na de diagnosestelling kan worden bepaald.

Met een steeds effectievere systemische therapie in het verschiet, zullen de huidige lokale behandelingsmodaliteiten, zoals chirurgie en radiotherapie, hun plaats in de multimodaliteits benadering van slokdarmkanker opnieuw moeten bepalen.

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CURRICULUM VITAE

Tjebbe Cornelis Kok is geboren op 4 februari 1950 te 's-Gravenhage. In 1969 behaalde hij het eindexamen gymnasium-beta aan het Johan van Oldenbarnevelt Gymnasium te Amersfoort.

Na een jaar studie Natuurkunde aan de Rijksuniversiteit te Groningen, werd in 1970 een aanvang gemaakt met de studie Geneeskunde, ook in Groningen. Na het artsexamen werden van 1977 tot 1979 wissel-assistentschappen gevolgd in de Interne Geneeskunde, Heelkunde, en Kindergeneeskunde in het Rooms-Katholiek Ziekenhuis te Groningen. Van 1979 tot 1980 werkte hij in de Werkgroep TNO/Epidemiologie van CARA mee aan het logitudinale bevolkingsonderzoek CARA Vlaardingen/Vlagtwedde.

Van 1980 tot 1985 volgde hij de opleiding Interne Geneeskunde op de afdeling Interne Geneeskunde II (hoofd: Prof.dr. M. Frenkel) van het Academisch Ziekenhuis Rotterdam / Dijkzigt. Vanaf 1985 is hij als internist, vanaf 1990 als internist-oncoloog, verbonden aan de afdeling Interne Oncologie van het AZR / Dijkzigt (hoofd: Prof.dr. G. Stoter). Hij is lid van de Rotterdamse Werkgroep voor Slokdarmtumoren, waarin naast de afdeling Interne Oncologie, ook de afdelingen Heelkunde, Inwendige Geneeskunde/Gastroenterologie, Radiotherapie, en Pathologische Anatomie van het AZR/Dijkzigt participeren.

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