CHEMOTHERAPY IN METASTATIC CANCER OF UNKNOWN PRIMARY

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Ate van der Gaast.
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CHEMOTHERAPY IN METASTATIC CANCER OF UNKNOWN PRIMARY

CHEMOTHERAPIE VOOR CARCINOMEN VAN ONBEKENDE ORIGINE

PROEFSCHRIFT

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	prof. dr. D.J.Th. Wagener
	prof. dr. Th.H. van der Kwast

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CHAPTER I

INTRODUCTION

1.1 Definition and diagnosis

In general a patient is considered to have a carcinoma of unknown primary site if no primary tumor can be identified after a thorough history and physical examination, and a reasonable laboratory and radiologic work-up. This definition also requires a histological diagnosis of carcinoma. The importance of histologic (re)examination in the management of patients with carcinoma of unknown primary site has been emphasized by several authors (1,2). Light microscopic examination may already provide a clue to suggest the site of origin (3). Immunohistochemistry and/or electron microscopy are of additional value, particularly in patients with undifferentiated tumors. A substantial number of patients with a light microscopic diagnosis of anaplastic tumor or undifferentiated carcinoma ultimately prove to have a lymphomas (4,5).

There is more debate about what should be considered as a reasonable work-up. Accepted basic evaluations are a chest x-ray, routine blood morphology and chemistry tests, visualization of the female pelvic organs, and determination of some tumor markers such as prostate-specific antigen, beta-human chorionic gonadotrophin or alpha-fetoprotein.

The yield of extensive radiographic or endoscopic evaluation is low and should not routinely be advocated (3, 6-10). As a general rule the work-up of patients with carcinomas of unknown primary site should be as limited as possible, guided by the presenting signs and symptoms of the disease and to identify patients with treatable tumors. For example, abdominal CT scan will enable the detection of primaries (11,12), but the detection of pancreatic cancer is irrelevant in the presence of metastases. Likewise, mammography may be helpful in women with axillary lymph node metastases only, but the overall yield in female patients with metastases of unknown primary is low (9).

1.2 Incidence

The precise incidence of carcinomas of unknown primary site is unknown. Not only have some tumor registries no heading for this entity, but the discrepancy in reported incidence is also related to different definitions. The incidence may be based on the diagnosis at first presentation when no primary tumor is found, or may be established at autopsy. Some authors consider only those patients whose primary remains hidden at postmortem examination as having a carcinoma of unknown primary site. Most reports on incidence reflect the incidence at first presentation after a diagnostic work-up. The reported incidence then varies from 0.5% to 15% (6, 13-17). Of note, the higher incidences are most often reported by cancer referral centers and are probably influenced by selection bias.

1.3 Histologic subtypes and presenting sites of disease

In the more than one million cases of cancer diagnosed in residents of SEER (surveillance, epidemiology, and end results) areas, about 2% were designated as being cancers of unknown primary site over a 15-year period of 1973-1987 (18). Approximately 55% of the 25,050 patients with histologically diagnosed cancers of unknown primary site had adenocarcinomas. About 20% had carcinoma not specified or undifferentiated, and about 10% had squamous cell carcinoma. Over the years a decline in the frequency of cancers of unknown primary has been noticed, probably due to advances in diagnostic methods. In a histology review of 343 patients with carcinoma of unknown primary site, Huebner found that 40% had adenocarcinomas, 28% undifferentiated carcinomas and 14% had squamous cell carcinomas (19).

In a site of metastatic involvement analysis of 657 patients reported by Abbruzzese, lymph nodes (37%), liver (31%) and bone (28%) were the predominant sites (20). In most studies, the predominant metastatic sites are lymph node, lung, bone, and liver (17). Many patients have tumor localizations in more than one organ site. The cervical lymph nodes are the most commonly involved anatomical site in patients

with squamous cell carcinomas (13,21).

1.4 Postmortem examinations

In many patients with carcinoma of unknown primary site, the primary is not identified because the primary is too small or because the appropriate clinical examinations were not performed. It is therefore most interesting to look at the results of postmortem examinations. Some studies report about small series so that no firm conclusions can be reached about the relative frequencies of the identified primary sites. Le Chevalier et al. (17) studied 302 autopsied patients with a carcinoma of unknown primary at presentation. During follow-up in 82 of these 302 patients the primary tumor was identified. The primary was found at autopsy in 173 patients and was not found in the in the remaining 47 patients (16%). In most cases the primary was localized in the pancreas, lung, prostate or adrenals. In the study of Didolkar et al. (14) autopsies were performed on 97 patients and the primary could be identified in 71 (73%). Most primaries were detected in the lung (40%), followed by stomach, pancreas, kidney, ovary and colon. Sherman and Garnick (22) combined 432 patients from several series in which the primary site was eventually found. Lung cancer (25%) and pancreatic cancer (17%) were the most commonly found origins. Other frequently encountered primary sites were colon, hepatobiliary system and stomach. The primary is more frequently found at autopsy in patients with adenocarcinomas than in patients with undifferentiated carcinomas (23).

1.5 There is no generally accepted classification system for carcinomas of unknown primary site

In the last century an accurate system has been developed for the classification of malignant tumors. This classification system is based on the primary origin of the tumor, the morphologic (microscopic) features of the tumor, and stage of the disease. Stage of disease is usually expressed according to the TNM classification, whereby T stands for the primary tumor size, N for the nodal status, and M for distant metasta-

ses. Both clinically and pathologically the T and N staging have been well developed and defined, since they have a strong predictive value for the risk of local recurrence and for the development of metastatic disease. By clustering TNM categories (stage grouping) it is often possible to identify groups with significant differences in survival.

The above mentioned classification system is, however, not applicable to patients with carcinomas of unknown primary site. Since in medical oncology predictions about tumor behavior and determination of appropriate therapy commonly are based on the primary tumor site and stage of disease, the absence of a primary tumor poses therapeutic problems and creates uncertainties in patient and physician (3). In an attempt to identify the primary the physician and the patient may demand extensive examinations which in the majority of cases do no alter the prognosis and management of the patient (7,8).

1.6 Natural history and prognostic factors

Overall median survival times reported for patients with carcinomas of unknown primary site are in the range of 3 to 6 months (1, 24). In an attempt to identify subgroups with a better prognosis, several investigators have performed analyses on prognostic factors. Abbruzzese analyzed 657 patients with carcinomas of unknown primary site (20). In a univariate analysis lymph node involvement, histologic diagnosis of squamous carcinoma and neuroendocrine carcinoma had a positive effect on survival. Male sex, diagnosis of adenocarcinoma, metastatic involvement of liver, bone, pleura or brain, and an increasing number of involved organ sites were variables negatively related to survival. In a multivariate analysis lymph node involvement, peritoneal carcinosis, and neuroendocrine histology were favorable prognostic factors. Male sex, an increasing number of involved organ sites, adenocarcinoma histology, and hepatic involvement had a negative effect on survival.

Earlier studies had already identified some of the above

mentioned prognostic factors either in selected patients with carcinomas or adenocarcinoma of unknown primary site. In a retrospective study of 254 patients Didolkar found that patients with metastatic squamous cell carcinoma with middle and upper lymph node involvement had the most favorable survival (14). In a series of 245 patients with metastatic adenocarcinomas of unknown primary site Markman found that age, sex, race and year of diagnosis did not appear to influence survival. However, patients with their major sites of disease above the diaphragm experienced a significantly longer survival than patients who had tumor below the diaphragm (25). Snee reported about 319 patients with metastatic carcinoma from unknown primary site. Patients with a good performance status and disease in only one organ had a significantly better survival than patients with a poor performance status and disease in multiple organs (15). In the study of Altman, involving 1539 patients, age below 35 years and histologic features of squamous cell carcinoma were factors associated with a more favorable prognosis (24). In a study of 286 patients with metastatic adenocarcinoma or undifferentiated carcinoma of unknown primary site, reported by Kirsten, factors independently predicting improved survival were lymph node presentation, good performance status and body weight loss less than 10% (9).

1.7 Chemotherapy trials

Most authors emphasize the fact that carcinoma of an unknown primary site is not a distinct disease entity, but constitutes a heterogeneous group of tumors that differ with respect to origin, biologic behavior, and prognosis. Nevertheless many chemotherapy trials have been performed in unselected groups of patients with carcinomas of unknown primary site. An overview of chemotherapy trials without cisplatin and with cisplatin is presented in Tables 1 and 2, respectively.

A comparison of the results of these trials is hampered by the fact that patients with a variety of histologic subtypes, variable stage of disease, and patient characteristics

were entered in these trials. In addition, about half of the trials did not meet the criteria of adequate sample size for phase II trials. Superficially, there does not seem to be a difference in treatment outcome with or without cisplatin. Considering the overall low response rates and brief survival many physicians offer these patients best supportive care only. However, a potential benefit of systemic treatment for a small subset of patients can not be detected from these trials.

Table 1

Chemotherapy trials without cisplatin in patients with carcinomas of unknown primary site

Year	Agent	No. of patients	Response rate	Median survival (all patients)	Ref.
1964	5FU	65	6%	-	27
1972	5FU	88	16%	-	28
1972	5FU,BCNU	11	18%	-	28
	5FU,M	7	0%	-	28
1979	5FU,A,C	14	14%	7+ mo.	29
1980	A,M	25	36%	4 mo.	15
	C,M,5FU	22	5%	2 mo.	
1982	5FU	33	-	3 mo.	25
1983	5FU,A,V	20	50%	8 mo.	21
1983	C,A,5FU	16	0%	3 mo.	31
	5FU	20	0%	4 mo.	
1985	A,Vds	38	16%	< 8 mo.	32
1986	5FU,A,M	28	22%	-	33
1986	5FU,A,M	43	30%	> 10 mo.	34
1987	5FU,C,V,Mtx	21	48%	-	35
1988	5FU,A,M	22	11%	8+ mo.	36
1989	5FU,A,M,Mtx	19	37%	15 mo.	37
1989	5FU,D,V,BCNU	61	20%	4 mo.	38
1990	M,A,V	57	30%	7 mo.	39
1992	5FU,Mtx,LV,PALA	21	5%	2 mo.	40
1993	5FU,LV	17	0%	5 mo.	41
1994	5FU,Inf,LV	6	50%	-	42

Abbreviations: 5FU = 5-Fluorouracil, BCNU = carmustine, M = mitomycin-C, A = doxorubicin, C = cyclophosphamide, V = vincristine, Vds = vindesine, Mtx = methotrexate, D = dacarbazine, LV = folinic acid, PALA = N-phosphonacetyl-l-aspartate, Inf = interferon alpha.

Table 2

Chemotherapy trials with cisplatin in patients with carcinomas of unknown primary site

Year	Agent	No. of patients	Response rate	Median survival (all patients)	Ref.
1983	DDP,A,C	13	15%	-	43
1986	5FU,A,M	7	14%	7 mo.	44
	5FU,A,M,DDP	35	26%	7 mo.	
1987	M,A	28	14%	5 mo.	45
	DDP,M,A	27	26%	5 mo.	
1987	M,A	51	42%	4 mo.	46
	DDP,Vlb,B	50	32%	6 mo	
1989	DDP,5FU,A,H	85	21%	6 mo.	47
1991	DDP	21	19%	5 mo.	48
1991	DDP,5FU,VP16	36	22%	11 mo.	49
1991	DDP,VP16	16	19%	7 mo.	50
1992	DDP,var	34	24%	4 mo.	51
	CBDCA,var	14	36%	4 mo.	
1993	DDP,5FU,LV	27	30%	18 mo.	52
1993	DDP,VP16,V,A,C	17	18%	5 mo.	53
	V,A,C	40	20%	5 mo.	

Abbreviations: DDP = cisplatin, CBDCA = carboplatin, 5FU = 5-Fluorouracil, VP16 = etoposide, M = mitomycin-C, A = doxorubicin, Vlb = vinblastine, B = bleomycin, C = cyclophosphamide, H = hexamethylmelamine, LV = folinic acid, V = vincristine, var = various other drugs.

1.8 Treatment strategies

The main issue in the treatment of patients with carcinomas of unknown primary site is to identify subgroups with treatable tumors. Patients are considered to have treatable tumors when there is a substantial chance of palliation and/or improved survival.

There are currently two approaches for the selection of these patients. The first one is based on the recognition of a metastatic pattern similar to the metastatic pattern of a known treatable primary tumor and to treat these patients in an identical way. Well known examples are: 1) women with axillary lymph node metastases only (breast cancer-like); (2) women with peritoneal carcinomatosis only (ovarian cancer-like; (3) cervical lymph node metastases only (head and neck cancer-like). The second approach is to select patients with chemosensitive histological subtypes; for instance those with poorly differentiated neuroendocrine tumors.

Treatable tumors identified by similarities in the metastatic pattern of treatable tumors with known primaries

Patients with axillary adenopathy and normal mammography are in most instances treated as having breast cancer with positive lymphnodes. Feuerman et al. found that 0.5% of patients with breast cancer initially had a normal appearing breast and a palpable lesion in the axilla (54). After mastectomy in approximately half the patients who presented with an isolated axillary lymphnode metastasis a primary tumor could be detected (55). The reported 5-year survival of these patients is similar to that of stage II breast carcinoma patients (56-58). It is suggested that estrogen and progesterone receptor assays should be obtained and are of aid in the selection of treatment of these patients (59). However, in general practice this knowledge does not influence the management of these patients. It seems appropriate to treat these patients with adjuvant hormonal treatment or adjuvant chemotherapy (60).

Cervical lymph node metastases of an unknown primary site are also a well recognized subgroup. Up to 5% of patients with metastatic cervical carcinoma do not have a detectable primary site despite a proper work-up (61,62). In the upper and middle cervical nodes squamous cell carcinomas and undifferentiated carcinomas are the most frequently encountered histologies. In the lower cervical nodes and supraclavicular lymph nodes the

frequency of adenocarcinomas increases (63). Depending on the extent of lymph node involvement, the location of the nodes and treatment, 5-year survival rates range from 30 - 70% (62, 64-67). Most of these results relate to the treatment of squamous cell carcinomas and undifferentiated carcinomas in the upper and middle cervical nodes. When lower cervical nodes and supraclavicular nodes are included the survival rates decrease. This latter finding is not surprising since it can be expected that a number of patients with occult lung cancers and gastric cancers may have metastases in these sites. Treatment plans may include neck dissection, radiotherapy or a combination of these. The role of chemotherapy is not clear.

Peritoneal carcinosis is common in patients with ovarian cancer, gastrointestinal cancer, and sometimes breast cancer. In some patients with peritoneal tumor involvement a primary tumor is not obvious. When a papillary serous histology is encountered this entity is also called papillary serous peritoneal carcinoma of extra-ovarian origin or multifocal extra-ovarian serous carcinoma (68,69). These patients are usually treated in the same way as patients with advanced ovarian cancer, including cytoreductive surgery and cisplatin combination chemotherapy. The response to treatment and the prognosis of patients with papillary serous peritoneal carcinoma of extra-ovarian origin is quite similar to those of patients with advanced ovarian cancer (70-72). The optimal management of patients with peritoneal carcinosis not having a papillary serous histology is not clearly defined.

A drawback in the approach of treatment selection according to a recognizable metastatic pattern is that the metastatic pattern at diagnosis often is not the same as in patients with clinically apparent primary tumors. For example, the distribution of bone metastases in patients with carcinoma of unknown primary site, which subsequently appears to be lung, is one-tenth of the frequency of what is seen in the usual lung cancers (1).

Treatment selection according to histology

Poorly differentiated adenocarcinomas, undifferentiated carcinomas and poorly differentiated neuroendocrine carcinomas are two examples of histological subtypes of carcinomas of unknown primary, which are considered to be responsive to chemotherapy.

Neuroendocrine tumors are a heterogeneous group of tumors. On the one end of the spectrum are chemoresistant tumors such as paragangliomas and islet cell tumors and on the other end chemosensitive tumors such as small-cell lung cancer. All these tumors share neuroendocrine features. Tumors with a small-cell carcinoma histology can arise in various organs and their biologic behavior and response to treatment is frequently alike the pulmonary counterpart (73,74). In our own series of 11 patients with extrapulmonary small-cell carcinomas nine patients had a response to chemotherapy. All 3 patients with an extrapulmonary small-cell carcinomas of unknown primary origin achieved a complete response (73). Moertel treated 45 patients with metastatic neuroendocrine tumors with a combination of cisplatin and etoposide (75). Only 2 (7%) out of 27 patients with well-differentiated carcinoid tumors or islet cell tumors responded to treatment. In contrast, 12 (67%) of 18 patients with anaplastic neuroendocrine carcinomas achieved a response. In a group of 29 patients with poorly differentiated neuroendocrine tumors of unknown origin treated at the Vanderbilt University 18 of 23 (78%) patients responded to chemotherapy (76,77).

The value of chemotherapy in a subset of patients with a histological diagnosis of poorly differentiated adenocarcinomas or undifferentiated carcinomas has been reported by Greco and Hainsworth (2, 78, 79) and is also the topic of some of the following chapters.

Even with the above described approaches a large number of patients do not clearly benefit from treatment. For example, only about half of the patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas respond to cisplatin containing chemotherapy and only a small proportion

of these patients achieve a durable response. Therefore, we need to identify prognostic factors more accurately, to select those patients who will benefit from treatment.

1.9 Scope of the thesis

The following chapters reflect the development of the management in the Western world of patients with carcinomas of unknown primary site. In chapter 2 the standard approach was to treat all patients with adenocarcinomas of unknown primary site with anthracycline and 5-fluorouracil containing chemotherapy in the contention that most of these patients had primaries located in the lung or pancreas and thus might benefit from such a treatment. Because of the low response rate obtained with this regimen, we deemed it necessary to develop new treatment approaches. The premise in chapter 3 was that there is no standard therapy for patients with well- and moderately differentiated adenocarcinomas of unknown primary site. These patients can be treated with either supportive care or with investigational drugs. In this chapter the use of etoposde is evaluated. In chapter 4 the results of cisplatin combination chemotherapy in a subset of patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas of unknown primary site are reported. A proportion of patients may achieve a durable complete response. In chapter 5 and 6 we attempted to identify more accurately those patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas who respond to chemotherapy. The value of immunohistochemistry in relation to prognosis in poorly differentiated adenocarcinomas or undifferentiated carcinomas is described in chapter 5. Finally, in chapter 6 an analysis of prognostic factors is presented which provides a model for the prediction of patients who will benefit from treatment.

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CHAPTER 2

5-FLUOROURACIL, DOXORUBICIN AND MITOMYCIN C (FAM) COMBINATION CHEMOTHERAPY FOR METASTATIC ADENOCARCINOMA OF UNKNOWN PRIMARY

Ate van der Gaast, Jaap Verweij, André S. Th. Planting and Gerrit Stoter.

Department of Medical Oncology, Rotterdam Cancer Institute, The Netherlands.

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ABSTRACT

The prognosis of patients with adenocarcinoma of unknown primary (ACUP) is dismal. Various chemotherapy regimens have yielded disappointing response rates and survival. Based on a report of Goldberg et al. (J Clin Oncol 1986, 4, 395-399) we performed a phase II study with 5-fluorouracil, adriamycin and mitomycin (FAM). Only three out of 22 evaluable patients achieved a partial response (14%) for a duration of 22, 30 and 74+ weeks. Median survival was 54+ weeks (range 35 - 74+ weeks) for responding patients and 33+ weeks (range 9 - 74+ weeks) for all treated patients. One patient (5%) developed mitomycin C induced hemolytic uremic syndrome. FAM cannot be recommended for routine use in patients with ACUP.

INTRODUCTION

The prognosis of patients with metastatic adenocarcinoma of unknown primary (ACUP) is poor. In a retrospective review of 245 patients, Markman reported a median survival of 3.1 months (1). At autopsy the primary tumor can only rarely be identified, mostly in the pancreas, the stomach or in the lungs (2).

Objective response rates of 0 - 16% have been reported with 5-fluorouracil as a single agent (3 - 5). Combination chemotherapy including adriamycin yields response rates of 0 - 50% (5 - 8) usually of short duration. A regimen including 5-fluorouracil (5-FU), adriamycin (ADM) and mitomycin C (MMC) (FAM) has been recommended for these patients because it has shown activity in patients with metastatic gastric cancer (9), adenocarcinoma of the pancreas (10) and adenocarcinoma of the lung (11), while a study of Goldberg et al. (12) resulted in a 30% response rate in patients with ACUP. To try to confirm the latter results we performed a phase II study with the same FAM regimen, in patients with ACUP.

MATERIALS AND METHODS

Twenty-three patients with measurable lesions of metastatic adenocarcinoma of unknown primary, 19 histologically proven and four cytologically proven, were treated with combination chemotherapy consisting of 5-FU, ADM and MMC. The FAM regimen was administered in 8 weeks cycles by i.v. bolus. 5-FU was administered at a dose of 600 mg/m² on days 1, 8, 29 and 36; ADM at a dose of 30 mg/m² on days 1 and 29; and MMC at a dose of 10 mg/m² on day 1 of each course. Treatment was discontinued for either progression or toxicity.

The minimum diagnostic evaluation for all patients included history and physical examination. chest X-ray, blood morphology and biochemistry, mammography in females and determination of serum acid phosphatase in men. Additional investigations are shown in Table 1.

Patients with bone lesions, ascites or pleural effusion as the only metastatic lesions were not considered eligible, as were patients with previous radiotherapy to the sole indicator lesion.

Patients were evaluated for response after each course of chemotherapy. However, patients with rapidly progressive disease after 4 weeks of treatment were documented as progressive disease.

Tumor response was evaluated by standard WHO criteria (13); complete response was defined as the disappearance of all known disease for at least 4 weeks; partial response as a more than 50% decrease in the sum of the greatest perpendicular diameters of all measurable lesions; stable disease as a less than 50% decrease and a less than 25% increase in measurable or evaluable lesions; and progression as an increase greater than 25% in the size of one or more measurable or evaluable lesions or the appearance of new lesions. Duration of partial response and survival were calculated from the time of start of chemotherapy. No patient was previously treated with chemotherapy. Clinical characteristics of the 22 evaluable patients are shown in Tables 2 and 3.

Table 1. Additional investigations

Diagnostic procedure	No. of patients
CT scan abdomen	17
Abdominal ultrasound	12
Bone scan	10
Isotopic liver-spleen scan	9
Intravenous pyelogram	9
Thyroid scan	8
Barium enema	7
Upper GI series	7
Bronchoscopy	6
CT scan chest	4
Cystoscopy	3
Gastroscopy	1
Laparoscopy	1

RESULTS

Of the 23 patients, one patients was inevaluable because of an ischemic cerebral event 6 days after the start of treatment, which precluded further therapy. No complete responses were achieved. A partial response was observed in three patients (14%). In two patients the durations of response were 22 weeks and 52 weeks respectively. In the third patient duration of response and survival is 74+ weeks.

Stable disease was achieved in 10 patients (45%). Two of them are still alive after a follow-up of 44 weeks and 61 weeks. The duration of stable disease ranged from 10 weeks to 52+ weeks with a median of 23+ weeks. The survival of the patients with stable disease ranged from 11 weeks to 81 weeks with a median of 41+ weeks.

Table 2. Patient characteristics

Evaluable	22
Median age (years)	55.6 (range 31 - 75)
Sex	
male	14 (64%)
female	8 (36%)
Performance status (WHO)	
0	10 (45%)
1	9 (41%)
2	3 (14%)
Interval diagnosis/treatment	
overall (median)	12 weeks
no previous treatment (14)	4 weeks
previous radiotherapy (4)	24 weeks
Number of sites of metastases	
1	6
2	14
> 2	2

Table 3. Sites of presentation

	No. of patients
Liver	7
Lymph nodes	7
Bone	6
Lung	5
Pleural/pleural effusions	4
Retroperitoneal	3
Intra-abdominal	2
Skin	2
Mediastinal	2
Other	2

Of the 22 evaluable patients nine (41%) had progressive disease after one course of chemotherapy. The median survival of these patients was 17 weeks.

Grades 3 and 4 hematologic toxicity were observed in four patients (18%), two with leukopenia and two with thrombocytopenia. Septicemia was not observed. Alopecia occurred in all patients who received more that one cycle of chemotherapy. One patients with a partial response developed the hemolytic uremic syndrome (HUS) after the fifth course of FAM after a total dose of 50 mg/m^2. Chemotherapy was discontinued and only symptomatic treatment was given avoiding red blood cell transfusions. No renal biopsy was performed. The patient is still alive 17 months after starting treatment, 8 months after the first signs of HUS. Renal function is still deteriorating with a serum creatinine level of 411 μmol/l (normal < 120). In the remaining patients the FAM regimen was well tolerated and could usually be given on an outpatient basis.

DISCUSSION

An extensive search for a primary lesion in the absence of signs, symptoms or laboratory abnormalities has not been found useful in patients with ACUP (14). Newer techniques, however, may be helpful in diagnosis and treatment. Reviewing biopsies with special stains, immunofluorescence studies, surface marker analysis, hormone receptor assays and electron microscopy may all be needed to identify treatable or curable tumors (15). Especially in patients with lymph nodes as the only site of disease immunopathology often offers the possibility to distinguish certain anaplastic carcinoma from high grade lymphoma (16). Greco et al. (17) reported an objective response rate of 56% in patients with undifferentiated (adeno) carcinomas, predominantly in the midline, with cisplatin combination chemotherapy. A proportion of these patients are believed to have an extragonadal germ cell tumor. Apart from perhaps the above mentioned subset of patients prognosis of disseminated ACUP is poor and no effective chemotherapy is

available.

Goldberg et al. (12) reported an overall response rate of 30% with the use of the same FAM regimen as used in the present study. In 22 evaluable patients we observed response rate of only 14%, with a median duration of 42+ weeks.

Although the FAM regimen is well tolerated concerning subjective and hematologic toxicity the risk of the MMC induced hemolytic uraemic syndrome (HUS) increases with the cumulative dose of MMC (18). Indeed one of our patients developed HUS. This patient had a relative long survival after the first signs of HUS, but continued to have deteriorating renal function, despite cessation of chemotherapy and avoidance of red blood cell transfusions.

We believe that combination chemotherapy in ACUP should be reserved for selected patients with a good performance status and in an experimental setting. For the remaining patients those measures should be offered that provide the best palliation to increase meaningful survival.

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CHAPTER 3

PHASE II STUDY OF ORAL ADMINISTRATION OF ETOPOSIDE FOR PATIENTS WITH WELL AND MODERATELY DIFFERENTIATED ADENOCARCINOMAS OF UNKNOWN PRIMARY SITE

A. van der Gaast[1], S.C. Henzen-Logmans[2], A.S.Th Planting, G. Stoter[1] and J. Verweij[1], .

[1]Department of Medical Oncology Rotterdam Cancer Institute - Daniel den Hoed Kliniek, and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands.
[2]Department of Pathology, Rotterdam Cancer Institute - Daniel den Hoed Kliniek, Rotterdam, The Netherlands.

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ABSTRACT

Background: The prognosis of patients with well- and moderately-differentiated adenocarcinomas of unknown primary is poor, as a consequence of chemotherapy resistance.

Patients and methods: We performed a phase II study with prolonged oral administration of etoposide in 25 chemotherapy-naive patients with well- and moderately-differentiated adenocarcinomas of unknown primary site. The treatment regimen was 50 mg/m² for 21 days, every four weeks.

Results: Of 24 evaluable patients, two achieved partial responses (8%) lasting 15+ and 17 months, 11 patients had stable disease, and 11 progressed during treatment. The major toxicity was myelosuppression. WHO grade 3 or 4 leukocytopenia was seen in six patients but only confined to the first treatment cycle in five of them. Four of these latter five patients already had disease progression after one treatment cycle. A primary tumor site was later identified in four patients, three colon carcinomas and one carcinoma of the pancreas.

Conclusion: Etoposide given in this dose and schedule has only limited activity in patients with well- or moderately-differentiated adenocarcinomas of unknown primary site.

Key words: carcinomas of unknown primary, chemotherapy, etoposide

INTRODUCTION

There is controversy as to whether (adeno)carcinoma of unknown primary constitutes a distinct clinical entity or merely underscores our diagnostic inability to detect a primary site. Although in general the prognosis of patients with carcinomas of unknown primary site can be regarded as poor with a median survival of 4 - 6 months, some of them may survive for more than one year after diagnosis (1). Moreover, in recent years a number of subgroups have been identified,

such as young patients with rapidly growing poorly-differentiated carcinomas and patients with poorly differentiated neuroendocrine tumors in whom high response rates to combination chemotherapy and even cure can be achieved (2-4).

For patients with well- and moderately-differentiated adenocarcinomas there is currently no standard chemotherapy.

Here we report the results of a phase II study with prolonged administration of oral etoposide in patients with well- and moderately-differentiated adenocarcinomas of unknown primary site.

PATIENTS AND METHODS

Patients with well- and moderately-differentiated adenocarcinomas of unknown primary site (3) were entered in the study. Eligibility requirements included measurable or evaluable disease, no prior chemotherapy, WHO performance status ≤ 2, leukocyte count $\geq 3000/\mu L$, platelet count $\geq 100,000/\mu L$, serum creatinine ≤ 150 μmol/L, serum bilirubin ≤ 20 μmol/L, a life expectancy of at least 8 weeks, and informed consent. Treatment consisted of oral etoposide administered as a singly daily dose of 50 mg/m^2 for 21 consecutive days, every four weeks. Complete blood counts were performed weekly. Patients were evaluated for response after every two courses. Those with rapidly progressive disease after one course of chemotherapy were classified as having progression. Tumor response and toxicity were evaluated by standard WHO criteria (5).

RESULTS

Patient characteristics are shown in Table 1. Of the 25 entered patients, one patient was not evaluable for response and toxicity, having died at home of unknown cause 15 days after the start of treatment.

Two of the 24 evaluable patients achieved partial responses lasting 15+ and 17 months. Their survivals were 15+

and 28+ months, respectively. One of these two patients initially presented with multiple liver metastases and a lymph node metastasis in the left axilla. After a response duration of 17 months, she progressed and underwent laparotomy because of bowel obstruction. She had a carcinoma of the colon. The second of the patients who achieved partial response had a solitary metastasis adjacent to the urinary bladder and is still in remission. Stable disease was observed in 11 patients (46%), with a median duration of 5 months (range 3 - 26 months) and eleven patients had progressive disease.

Table 1. Patient characteristics.

No. patients	25	
Sex		
Female	15	(60%
Male	40	(40%)
Median age (years)	60	
Range	41 - 71	
Median performance (WHO)	1	
Range	0 - 2	
Histology		
Well-differentiated adenocarcinoma	1	(4%)
Moderately-differentiated adenocarcinoma	24	(94%)
Prior radiotherapy	1	
Prior hormonal therapy	2	
No. sites		
1	16	(64%)
2 or more	9	(36%)
Sites of disease		
Liver	18	
Lung	4	
Bone	3	
Lymph nodes	8	
Other	5	

Four of the five patients who developed grade 3-4 leukocytopenia during the first course also had evidence of disease progression after one cycle and received no further treatment. During the succeeding courses of treatment (total number = 62) a grade 3 or 4 leukocytopenia was observed only once. Granulocytopenic fever occurred in two patients. Thrombocytopenia was not observed. A WHO grade 1 or 2 anemia was observed in 12 patients and a WHO grade 3 anemia in one patient. Gastrointestinal toxicity was absent (50%) or mild (46%) in most patients. Only one patient (4%) had prolonged nausea and vomiting. Alopecia was common. The primary tumor site were later identified in four patients: Three had colon carcinoma and one patient had a carcinoma of the pancreas. In three of these patients the primary site was identified at autopsy.

DISCUSSION

Etoposide has demonstrated considerable efficacy against a broad spectrum of tumors (6). Studies on the oral prolonged use of etoposide are scanty and have yielded a maximum tolerated dose of 50 mg/m^2 per day for 21 consecutive days as well as responses in several solid tumor (7).

We investigated the activity of a 21-day schedule of oral etoposide in patients with moderately- and well-differentiated adenocarcinomas of unknown primary. Leukocytopenia was predominantly seen in patients with progressive disease after one course of treatment. In 54% of the patients leukocytopenia was absent or confined to WHO grade I. Considering the variable oral absorption of etoposide it is possible that a number of patients were under-dosed (8). The non-hematologic toxicity was mild, only one patient had protracted nausea. In view of the observed response percentage of 8.3% (95% confidence interval 0 - 20) and the median survival of 7 months, it can be concluded that etoposide given in this schedule has very limited activity.

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CHAPTER 4

CARCINOMA OF UNKNOWN PRIMARY: IDENTIFICATION OF A TREATABLE SUBSET?

A. van der Gaast[1,3], J. Verweij[1], S.C. Henzen-Logmans[2], C.J. Rodenburg[1] and G. Stoter[1].

Departments of [1]Medical Oncology and [2]Pathology, Rotterdam Cancer Institute.
Department of [3]Medical Oncology, University Hospital Rotterdam-Dijkzigt, The Netherlands.

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SUMMARY

We initiated a phase II study with combination chemotherapy consisting of cisplatin, etoposide and bleomycin in a subset of patients with carcinomas of unknown primary site characterized by the presence of at least one of the following criteria: 1) age below 50 years; 2) clinical evidence of rapid tumour growth; 3) tumour located predominantly in a midline distribution; 4) good response to previous administered radiotherapy. In 34 evaluable patients an objective response rate of 53% (95% confidence limits 35-70%) was achieved. For patients with poorly differentiated adenocarcinomas the response rate was 35% and, in most instances, of short duration. A response rate of 79% including complete responses and long-term survivals was achieved in patients with undifferentiated carcinomas. This difference in response rate was statistically significant ($p = 0.02$). No supplementary prognostic factors predicting response to chemotherapy could be identified. One patient with an initial diagnosis of undifferentiated carcinoma proved to have a malignant lymphoma after additional immunohistochemical investigation. Until a better characterization of this syndrome is possible patients with undifferentiated carcinomas of unknown primary site should be challenged with cisplatin-based chemotherapy.

KEY-WORDS: Carcinoma unknown primary, chemotherapy.

INTRODUCTION

The number of patients seen in cancer referral centers with metastatic carcinomas of unknown primary does not seem to decrease substantially despite improved imaging and endoscopic diagnostic methods and the application of immunohistochemistry and serum tumour-markers. Even at postmortem examination the primary site is seldom ascertained (1).

Although patients with carcinomas of unknown primary are

believed to constitute a heterogeneous group, most of them present with a far-advanced malignancy and a limited survival of 2 - 6 months (2). The grim prognosis of these patients, refractory to all previously tested chemotherapeutic regimens (3-5), explains the nihilistic approach of many physicians.

One of the means for overcoming this problem is to identify subgroups of patients most likely to benefit from treatment. By identification of prognostic factors the disappointing results of many trials on treatment might perhaps be averted.

In the group as a whole with advanced poorly differentiated tumours of unknown primary, Greco et al. claimed to recognize a treatable syndrome (6). An objective response rate of 56% and a complete response rate of 22% were reported in a group of 71 patients with one or more clinical features of the extragonadal germ cell cancer syndrome who received a cisplatin-based regimen. It therefore seemed worthwhile to investigate whether in a prospective study we could also recognize such a syndrome and identify further prognostic factors for response to chemotherapy. A combination chemotherapy regimen consisting of cisplatin, etoposide and bleomycin was employed because this combination is known to be more active in teratocarcinomas and seminomas and is less toxic than the regimen of cisplatin, vinblastine and bleomycin most often used by Greco (6).

MATERIALS AND METHODS

In this study patients were considered to have a carcinoma of unknown primary site if no primary site could be identified after a thorough history and physical examination, a chest X-ray, routine biochemical and hematological studies, a mammogram for women, visualization of the female pelvic organs and an assessment of tumour markers such as acid phosphatase, ß-HCG and α-fetoprotein. Patients with a histological diagnosis of adenocarcinoma or undifferentiated carcinoma were ente-

red in the study provided they had at least one of the following features: 1) age under 50 years (particularly men); 2) tumour located predominantly in a midline distribution (mediastinum or retroperitoneum), multiple pulmonary nodules or lymphadenopathy; 3) clinical evidence of rapid tumour growth; 4) tumour very responsive to previously administered radiotherapy.

Table 1. Additional investigations

Diagnostic procedure	No. of patients
CT scan abdomen	32
Abdominal ultrasound	28
Bone scan	16
CT scan chest	15
Gastroscopy	15
Thyroid scan	12
Bronchoscopy	9
Barium enema	9
Laparoscopy	6
Sigmoido/colonoscopy	6
Intravenous pyelogram	6
Upper GI series	5

The histological material of all patients was reviewed and additional immunohistochemistry to exclude other, possible treatable malignancies was performed. Patients with an apparent diagnosis of extragonadal germ cell cancer were not included in this protocol. Additional investigations of the eligible patients are shown in Table 1.

The patients received a combination chemotherapy consisting of cisplatin 20 mg/m²/d, days 1 - 5, etoposide 120 mg/m² days 1, 3, and 5, and bleomycin 30 mg on days 2, 9, and 16.

Cycles were repeated every three weeks for at least four cycles, provided no progression or intolerable toxicity occurred. Patients still responding after the fourth cycle of chemotherapy were treated with two additional cycles of etoposide and cisplatin.

Patients were evaluated for response at administration of every other course. However, those with rapidly progressive disease after one course of chemotherapy were recorded as having progression. Tumour response and toxicity were evaluated by standard WHO criteria (7). Duration of partial response and survival were calculated from the time of start of chemotherapy. Duration of complete response was calculated from the date the complete response was first recorded to the date on which progressive disease was noted.

RESULTS

Of the 42 patients treated, two were ineligible, one because of a histological diagnosis of squamous cell carcinoma and the other because of an absence of measurable parameters. At present 34 patients are evaluable for response, it is too early for four, one patient died early due to a cardiovascular event two days after the start of treatment, and one patient refused further treatment after the first cycle of chemotherapy.

Of the evaluable patients 20 had histological diagnoses of poorly differentiated adenocarcinoma characterized by rudimentary gland formation and/or intracellular lumina with evidence of mucin production, and 14 patients had histological diagnoses of undifferentiated carcinoma. Clinical characteristics are shown in Table 2.

Four patients (12%) achieved CRs, with a median duration of 4, 5, 20+, and 21+ months; their survival ranged from 7+ to 21+ months. All 4 had histological diagnoses of undifferentiated carcinoma. Two of these patients, one of whom developed cerebral metastases with no further evidence of disease, have

relapsed.

Partial responses were observed in 14 patients (41%). Seven patients with PRs had poorly differentiated adenocarcinomas and 7 undifferentiated carcinomas. The duration of the PRs ranged from 3 to 12+ months, with a median of five months. The survival of these patients ranged from 3 to 19+ months, with a median of 8+ months. Stable disease was achieved in 12 patients (35%), with a median duration of 4 months (range 2 - 12+ months) and a median survival of 6 months. The median survival of the 4 patients with progression all of whom had histological diagnoses of adenocarcinoma was 2 months (range 1 - 3 months). In patients with objective responses all relapses occurred at the initial tumour sites with the exception of the one patient who developed cerebral metastases after having achieved a complete response.

Table 2. Patient characteristics

Evaluable	34
Median age (years)	51 (range 21-63)
Sex	
male	19
female	15
Performance status (WHO)	
0	13
1	15
2	6
Interval diagnosis/treatment	1 month (median)
Previous chemotherapy	0
Previous radiotherapy	8
Number of sites of metastases	
1	16
2	11
>2	7

Table 3 lists the number of responses for such distinctive criteria as midline presentation, rapid growth, age below 50 years and according to respective histology.

Hematologic toxicity consisted of grade 3 leukopenia in 36% and grade 4 in 18% of the patients, complicated in four patients by a successfully treated septicaemia. Seven patients developed grades 3 or 4 thrombocytopenia, and one patient died of a treatment-related cerebral haemorrhage. Nausea and vomiting occurred in all patients, and alopecia was common. Reversible grade 1 renal toxicity was observed in 12% of the patients, and a mild neurotoxicity was found in 24% at the end of treatment.

Bleomycin-induced skin changes were seen in 33% of the patients, and pulmonary changes, also induced by bleomycin, were found in 7 patients. In three of the latter 7 there was clinically overt interstitial pneumonitis which, in two of them, was successfully treated with corticosteroids. The third patient died of progressive respiratory insufficiency due to bleomycin-induced pneumonitis after the third cycle of chemotherapy (total bleomycin dose 270 mg.). Lung function and carbon monoxide diffusion capacity tests performed before the third cycle had revealed no abnormalities. At postmortem examination an extensive interstitial fibrosis was found in both lungs.

DISCUSSION

Patients presenting with an undifferentiated malignant tumour of unknown primary site are a problem frequently encountered in clinical oncology. In the management of these patients several specific questions have to be answered in order to identify patients with neoplasms potentially curable or amenable to effective chemotherapy or radiotherapy. In the case of undifferentiated malignancies the application of immunohistochemical techniques usually makes it possible to distinguish high-grade malignant lymphomas from undifferenti-

ated carcinomas (8-10). Almost all patients in the present study had tumours that were reactive to the anti-cytokeratin antibodies (CAM 5.2 and polyclonal anti-keratin) and did not react with the anti-leucocyte common antigen antibody (DAKO-LC), which makes the inclusion of patients with malignant lymphomas unlikely. However, one of the patients who was referred with an initial diagnosis of undifferentiated carcinoma and who achieved a complete response proved in fact, to have a malignant lymphoma after additional immunohistochemical investigation.

It is now generally accepted that an extensive radiological search in the absence of clinical signs or symptoms is unproductive and in some instances may even be misleading (11-12).

Table 3.

	CR	PR	SD	PD	Total
rapid growth	3	10	4	4	21
midline	3	9	9	0	21
age < 50 years	1	6	4	2	13
poorly differentiated adenocarcinoma	0	7	9	4	20
undifferentiated carcinoma	4	7	3	0	14

No. of responding patients according to features of disease. Abbreviations: CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease.

The success of postmortem examination in detecting the primary is correlated with the histological grade of the tumour (13). However, in a number of patients with a carcinoma of unknown primary, the primary site at autopsy can be identified in the pancreas, the lungs, the hepatobiliary system or

in the stomach, which explains the poor survival and response to treatment of these patients (11,14).

In patients with advanced poorly differentiated carcinomas of unknown primary site high response rates and long lasting complete responses were reported using cisplatin-based chemotherapy (15). Patients were especially prone to response if tumours were characterized by a short doubling time and a predominant involvement of the midline. In addition some patients had elevated serum levels of ß-HCG and/or α-FP at presentation and/or α-FP or ß-HCG demonstrated in the tumour cells by immunohistochemical methods (6,15). The question of whether these patients have an atypical germ cell tumour is still unanswered. Repeated biopsies at relapse after treatment showed some of the tumours to have histological features of a germ cell tumour. Further studies such as a comparison between the cytogenetic abnormalities found in these tumours and those found in extragonodal germ cell tumours should be undertaken.

With the exception of a single individual, none of the patients in this study had neoplasms which displaying α-FP and/or ß-HCG, or clearly elevated serum levels of α-FP and/or ß-HCG, in contrast to 23% of the patients reported by Greco. This finding also argues against the inclusion of germ cell cancers. The only patient whose tumour expressed ß-HCG had a stabilization of his disease for a period of 2 months.

No correlation could be found between response to treatment and/or survival and age or sex of the patients, the number of involved sites and the presence or absence of liver involvement.

Patients with poorly differentiated adenocarcinomas as well as those with undifferentiated carcinomas were included in our present study. In accord with the results of Greco, patients with adenocarcinomas did less well than those with undifferentiated carcinomas, with respective response rates of 35% versus 79%. Although the number of patients included in this study is still small, the difference between response rates of patients with adenocarcinomas and those with undif-

ferentiated carcinomas is statistical significant ($p = 0.02$). Interestingly, in 11 of the 14 patients with undifferentiated carcinomas the dominant site of disease involvement was the mediastinum, retroperitoneum or lymph-nodes, whereas in 10 of the 20 patients with adenocarcinomas the dominant site of disease was outside these areas and the lesions were more characterized by rapid growth. Only 3 of these patients responded to treatment, in contrast to 4 of the 10 patients with the dominant site of disease located in the midline.

A high incidence of 21% of pulmonary changes induced by bleomycin was found in this study. In most instances these changes were characterized by the development of basal crepitations or rales, a decrease in capacity for carbon monoxide diffusion or by the appearance of radiologic abnormalities in the chest X-ray. Three of these patients developed clinically overt interstitial pneumonitis, which in one patient led to a toxic death. The reason for the high incidence of pulmonary side effects remains obscure.

In view of the relatively low response rate of 35% and limited duration of the responses, it remains highly debatable whether patients with metastatic adenocarcinomas, even if the dominant site of disease is in the midline, should be treated with this intensive chemotherapeutic regimen. Patients with undifferentiated carcinomas of unknown primary site characterized by rapid tumor growth and/or predominant involvement of the midline should be challenged with cisplatin-based chemotherapy, because long lasting complete responses can be achieved in a number of these patients, and some may even be cured. However, the precise identification of these patients remains difficult.

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CHAPTER 6

THE VALUE OF IMMUNOHISTOCHEMISTRY IN PATIENTS WITH POORLY DIFFERENTIATED ADENOCARCINOMAS AND UNDIFFERENTIATED CARCINOMAS OF UNKNOWN PRIMARY

A. van der Gaast[1], J. Verweij[1], A.S.Th Planting, G. Stoter[1] and S.C. Henzen-Logmans[2].

[1]Department of Medical Oncology, Rotterdam Cancer Institute - Dr Daniel den Hoed Cancer Center, and University Hospital Rotterdam-Dijkzigt, Rotterdam, The Netherlands.
[2]Department of Pathology, Rotterdam Cancer Institute - Dr Daniel den Hoed Cancer Center, Rotterdam, The Netherlands.

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ABSTRACT

A subgroup of patients with metastatic carcinomas of unknown origin may benefit from combination chemotherapy. The relevance of immunohistochemistry in detecting such patients was investigated. Immunohistochemical studies with a panel of antibodies were performed on the tissue specimens of 41 patients having a light-microscopic diagnosis of poorly differentiated adenocarcinoma or undifferentiated carcinoma of unknown origin, who had been treated with cisplatin-containing chemotherapy. The study aimed to answer the following questions: (a) Can the tissue type of the tumor be verified? (b) Can a primary organ site be identified? (c) Can a prognostic immunohistochemical profile be recognized?. The original diagnosis had to be changed in 2 of the 41 patients, who turned out to have a malignant lymphoma and neuroblastoma, respectively. The primary site was diagnosed in a patient with prostate cancer, whereas in one case the diagnosis could be narrowed down to a neuroendocrine tumor. No certain immunohistochemical profile with prognostic significance could be identified. It was concluded that immunohistochemistry should be routinely used in cases of undifferentiated carcinoma of unknown primary origin to verify the histological diagnosis and to select the appropriate therapy.

Key words: Carcinoma of unknown origin, Immunohistochemistry, Chemotherapy

INTRODUCTION

Malignant tumors are traditionally classified according to site of origin, tissue type, and histological subtype. Consequently, a variety of tumors can be recognized, each with its specific biological behavior and prognosis. The choice of treatment is mainly related to the site of origin of the tumor and to the tissue type, especially where patients with meta-

static malignancies are concerned.

In 0.5% - 15% of the cases presenting with metastatic tumors the primary tumor site is not found, despite extensive diagnostic procedures (Holmes and Fouts 1970; Didolkar et al. 1977; Nystrom et al. 1979; Woods et al. 1980; Neumann and Nystrom 1982; Le Chevalier et al. 1988). The prognosis in these cases is generally poor due to poor response to chemotherapy.

Previous studies, both by us and by others, have shown that a subgroup of metastatic carcinomas are chemosensitive, and may therefore have a better prognosis in terms of response rates and survival (Hainsworth et al. 1988; van der Gaast 1990). Some of the characteristics of this subgroup are rapid tumor growth, young age, midline distribution of the tumor and poor histological differentiation. At the Vanderbilt University Medical Center an update on 220 patients with undifferentiated carcinomas or poorly differentiated adenocarcinomas of unknown primary site, treated with cisplatin-containing regimens, showed an objective response in 63% of the patients, with an actuarial 10-year survival of 16% (Hainsworth et al. 1992a).

The clinical utility of immunoperoxidase staining in patients with a light-microscopic diagnosis of undifferentiated carcinomas or poorly differentiated adenocarcinomas of unknown primary site was reported by Hainsworth and co-workers in 1991 (Hainsworth et al. 1991). In 14 patients (16%) out of a group of 87, immunohistochemistry led to a different diagnosis, including lymphoma and melanoma. In 24 patients the results were inconclusive, but a more specific diagnosis could be made in 7 out of 18 patients by means of electron microscopy performed on the tissue specimens.

This report describes an investigation of the relevance of immunohistochemical analyses performed on the histological specimens in a similar subgroup of patients. The aims of this study were:

1. To verify the light-microscopic classification of the

tissue type of the tumor.
2. To assess whether the application of a panel of antibodies contributes to the identification of a primary organ site.
3. To assess the prognostic significance of various immunohistochemical profiles.

MATERIALS AND METHODS

Immunohistochemical studies were performed on the tissue specimens of 41 patients with a carcinoma of unknown primary origin treated with chemotherapy.

Tumors were considered to be of unknown primary site if, after a thorough history and physical examination, chest X-ray, routine blood morphology and chemistry tests, mammography in women, palpation and visualization of the female pelvic organs and an assessment of tumor markers such as prostate-specific antigen, ß-human chorionic gonadotropin (HCG) and α-fetoprotein (AFP), no primary site could be identified.

The treatment regimen consisted of intravenous administration of 20 mg/m² cisplatin on days 1-5, 120 mg/m² etoposide on days 1,3, and 5, and 30 mg bleomycin on days, 2, 9, and 16. Four cycles were given at 3-week intervals.

Only patients with a light-microscopic diagnosis of poorly differentiated adenocarcinoma or undifferentiated carcinoma, meeting at least one of the following criteria, were entered (Greco et al. 1986):
1. Age under 50 years (particularly men)
2. Tumor predominantly located in a midline distribution (mediastinum or retroperitoneum), multiple pulmonary nodules or lymphadenopathy
3. Clinical evidence of rapid tumor growth.

A tumor was classified as a poorly differentiated adenocarcinoma when features like rudimentary gland formation and/or intracellular vacuoles with evidence of mucin production were present. If none of these characteristics was found, the tumor was classified as undifferentiated carcinoma.

Tumor response was evaluated by standard WHO criteria (WHO 1979). Duration of survival was calculated from the starting date of chemotherapy. Patient characteristics are listed in Table 1.

Table 1. Patient Characteristics.

No. patients	41
Sex	
Female	23 (56%)
Male	18 (44%)
Age (years)	
Median	50
Range	21 - 61
WHO performance status	
Median	1
Range	0 - 2
Histology	
Poorly differentiated adenocarcinoma	23 (56%)
Undifferentiated carcinoma	18 (44%)
Response	
Complete response	5 (12%)
Partial response	17 (41%)
Stable disease	12 (29%)
Progressive disease	7 (17%)

Immunohistochemistry

Immunohistochemical staining was performed on paraffin sections, using the indirect immunoperoxidase method. Staining results were recorded as either positive or negative for each case. A positive control section was included in each staining series. Phosphate-buffered saline, non-immune ascites fluid or normal rabbit immunoglobulin were used as negative controls (Henzen-Logmans et al. 1987).

In order to verify the tumor tissue type, the following

antibodies were selected in appropriate dilutions: monoclonal antibody (mAB) CAM 5.2 (cytokeratin 8, 18) (Becton Dickinson, USA); mAB 2662 (broad-spectrum keratin) (DakoPatts, Denmark); polyclonal pVi (vimentin) (Eurodiagnostics Organon Teknika, The Netherlands); mAB NKI/C3 against melanoma-associated antigen glycoprotein 90-34 (kindly provided by dr. Claus Vennegoor, NCI, Amsterdam, The Netherlands); DAKO-LCA (CD45) (DakoPatts, Denmark).

In order to identify the primary tumor site, the following tumor selective or tumor-associated antibodies were used: mAB CEA (carcinoembryonic antigen) (Eurodiagnostics Organon Teknika, The Netherlands); mAB OV632 against ovarian-associated antigen, (Monosan 9003, Sambio, Uden, The Netherlands); and MAB A577 (calcitonin), A251 (thyroglobulin), A008 (AFP), A231 (ßHCG), A627 (PZF), L1838 (PSA) all purchased from DakoPatts, Denmark; and mAB OC125 (HistoCIS, France).

Statistical methods

The following factors were analyzed for prognostic significance: histology and immunohistochemical staining for cytokeratins, vimentin, NKI/C3, CEA, OV632, calcitonin, thyroglobin, AFP, ßHCG, PZF and PSA. Survival curves were calculated according to the Kaplan-Meier method (Kaplan and Meier 1958) and differences between the survival curves were tested using the log-rank test (Mantel 1966). A P-value below 0.05 was considered to be significant.

RESULTS

Patients with a revised tissue type or definitive diagnosis

Of the 41 patients, 39 had tumors reactive to the anti-cytokeratin antibodies, which suggests epithelial differentiation of the tumor. The tumor of 1 of the 2 remaining patients, reacted with the anti-leucocyte common antibody (LCA). This patient was referred to our department with a diagnosis of

undifferentiated carcinoma but, after additional immunohistochemical studies, proved to have a malignant large-cell non-Hodgkin lymphoma. He had a complete response after four cycles of chemotherapy and, more than 5 years after the initial diagnosis, the patient is now disease-free. The other cytokeratin-negative patient had a tumor that only reacted with anti-vimentin. After additional electron-microscopy a tentative diagnosis of neuroblastoma was made. This latter patient achieved a partial response to chemotherapy for 12 months, and survived for 18 months. Apart from the one above-mentioned patient no other tumors reacted with anti-LCA.

One tumor (in a 45-year-old patient) reacted with L1838, which is directed against prostate-specific antigen (PSA). This patient presented with a poorly differentiated adenocarcinoma with bone metastases and rapidly growing lymph node metastases in the neck. Rectal examination was initially reported to be negative. On reception of the immunology results a histological biopsy of the prostate was performed, revealing a carcinoma of the prostate, which also turned out to be PSA-positive.

Patients with tumors positive for one or more tumor-associated antigens

In 1 patient the tumor stained with anti-calcitonin. This patient presented with rapidly growing liver metastases of unknown origin. There was no clinical evidence of a (medullary) carcinoma of the thyroid. The initial histological findings were compatible with a poorly differentiated adenocarcinoma. Additional staining revealed that this tumor also expressed neuron-specific enolase and chromogranine A, a reaction pattern characteristic for a neuroendocrine tumor. The patient did not respond to chemotherapy and died 3 months after start of treatment.

In the female patients, immunohistochemical studies with OV-632 and OC-125 monoclonal antibodies, directed against non-mucinous ovarian carcinomas, were performed. None of the

tumors expressed OV-632 and only one tumor reacted with OC-125. Post-mortem examination in this patient did not reveal any primary tumor, ovarian or otherwise.

The tumors of 2 patients were ßHCG-positive. One of these was the previously mentioned patient with prostate cancer. The other patient was a woman who presented with axillary and supraclavicular lymph node metastases of a poorly differentiated adenocarcinoma without any evidence of a germ cell tumor, apart from the immunology result. This patient partially responded to chemotherapy and was still alive 28 months after start of treatment without evidence of disease progression.

Immunohistochemical patterns requiring further investigations

In 17 patients the tumors stained with NKI/C3. NKI/C3 reacts with an antigen, glycoprotein 90-34, expressed in the cytoplasm of melanoma cells. However, like other melanoma-associated antigens, NKI/C3 reactivity may also be present in a variety of other tumors (Vennegoor et al. 1985). NKI/C3 often reacts with neuroendocrine tumors and also with mucin-producing tumors of the breast and lung. Especially degenerating cells are often NKI/C3-positive. In 3 patients the tumors stained with cytokeratins as well as with vimentin and NKI/C3. As melanomas normally express vimentin but may also express cytokeratin (Zarbo et al. 1990), additional studies were performed in these 3 patients. Electron microscopy performed on the material of 1 patient showed no premelanosomes or melanosomes. Additional staining for S-100 protein and melanin was negative in the 2 other cases. In none of the 17 patients could melanoma be established as the primary diagnosis.

Prognostic relevance of certain immunohistochemical profiles

A total of 16 patients had tumors that stained with anti-CEA. Of these 16 cases, 14 had a histological diagnosis of poorly differentiated adenocarcinoma. The clinical diagnosis

could not be narrowed down in any of these cases. No difference in survival (P=0.64) was observed between the 16 patients with CEA-positive tumors and the 25 CEA-negative ones. Likewise, no survival difference (P=0.51) was observed between the 7 vimentin-positive cases and the 34 vimentin-negative ones, or between the 17 patients with NKI/C3-positive tumors and the 24 NKI/C3-negative patients (P=0.08). The influence of the various parameters on response and survival is shown in Table 2. No discrepancies could be found in the immunoprofiles of the patients with a survival of less than 12 months, as opposed to those with a survival of more than 12 months after the start of treatment. The survival curve for all patients is shown in Fig. 1.

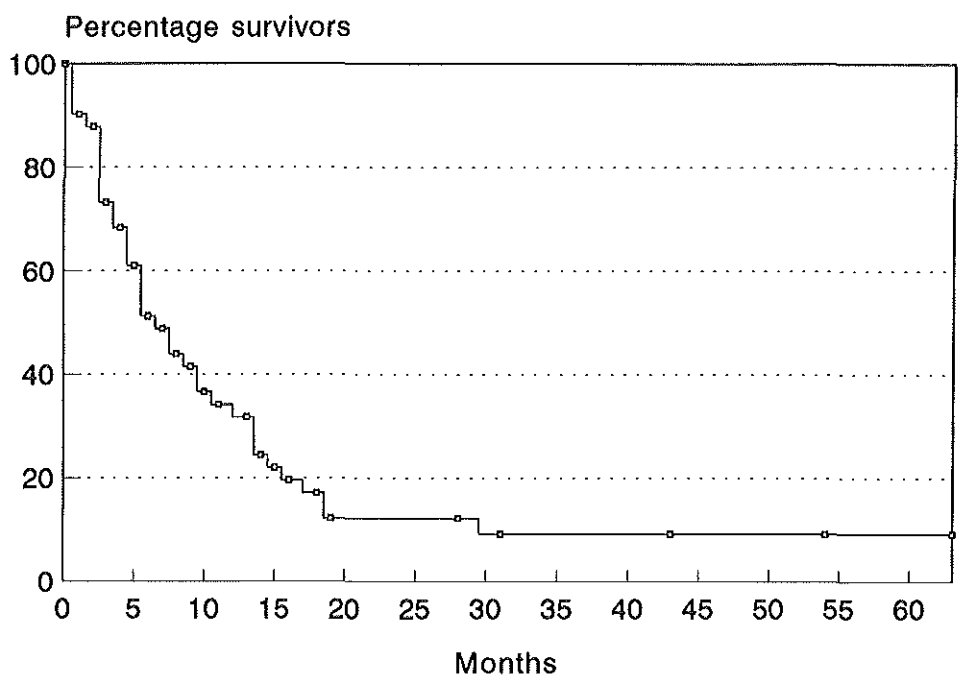

Fig. 1 Survival curve for all patients (n=41)

TABLE 2

Prognostic significance of histology and certain immunohistochemical profiles

Variable	Response rate	Median survival (months)	Significance
Histology			
adeno (n = 23)	39%	6	p = 0.23
undiff ca. (n = 18)	72%	9	
Vimentin-expression			
positive (n = 7)	57%	6	p = 0.49
negative (n = 34)	51%	8	
NKI/C3-expression			
positive (n = 17)	47%	5	p = 0.08
negative (n = 24)	58%	8	
CEA-expression			
positive (n = 16)	50%	7	p = 0.64
negative (n = 25)	56%	6	

DISCUSSION

Despite the development of sophisticated examination methods, such as computed tomography and magnetic resonance imaging, the detection of the primary site of a metastatic solid malignancy remains a problem. It is well known that patients with carcinoma of unknown primary site carry a poor prognosis, not only because the majority of them present with far advanced disease with extensive bone or liver metastases, but also because most tumors are refractory to drug therapy

(Ultmann 1991). However, a subset of patients does have a better prognosis after treatment with cisplatin-containing chemotherapy. These patients are characterized by features such as rapid tumor growth, young age, midline distribution of the tumor, and an undifferentiated histology (Hainsworth et al. 1988, 1992b; van der Gaast et al. 1990).

We have investigated the value of immunohistochemical analysis of metastatic tumor tissue in this subgroup of patients. As a result, in 2 patients (5%), the tissue diagnosis was changed to a malignant lymphoma and neuroblastoma respectively. In 1 patient with a poorly differentiated calcitonin-positive adenocarcinoma, the diagnosis could be refined to a neuroendocrine tumor. The primary organ site could be diagnosed in 1 patient with prostate cancer.

In the previously mentioned study of Hainsworth et al. the tissue diagnosis had to be changed in 17 of the 87 patients (20%), while in another 4 patients a diagnosis of the primary organ site or a diagnosis refinement could be made. The low percentage of changed diagnoses in our study may be due to the fact that the majority of our patients were referred from other centers, and that light-microscopic review pathology of the tissue specimens had taken place before the patients entered this study.

In the group of patients we studied no histological or immunohistochemical characteristics could be identified to discriminate between good or poor prognosis. Although the response rate to chemotherapy was significantly (P=0.03) higher in patients with undifferentiated carcinomas (72%) than in patients with poorly differentiated adenocarcinomas (39%), the survival curves of these two groups are similar. Greco et al. also found a higher response rate in patients with undifferentiated carcinomas compared to patients with poorly differentiated adenocarcinomas of unknown origin treated with cisplatin-containing chemotherapy. However, in a multivariate analysis, histology was not shown to have an independent predictive value for survival (Greco and Hainsworth 1990;

Hainsworth et al. 1992).

We found more adenocarcinomas than undifferentiated carcinomas to be CEA-positive, as was to be expected (Goslin et al. 1981), but CEA expression had no prognostic significance for either response or survival. Some reports suggest that vimentin expression may be a poor prognostic indicator (Raymond and Leong 1989; Domagala et al. 1990), but the patients' survival pattern in our study showed no correlation with vimentin expression. None of the tested antibodies, or combination of antibodies, appeared to yield a subgroup of patients with prognostic benefit.

The results of this study confirm that a pathology review, including light microscopy and immunohistochemistry, will elucidate the precise tissue and primary organ site diagnosis in about 5% of cases, and support the contention that immunohistochemistry with a restricted panel of antibodies, such as applied here, should be routinely used in cases of undifferentiated carcinoma of unknown primary origin to verify the histological diagnosis and to select the appropriate therapy.

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CHAPTER 6

A SIMPLE PROGNOSTIC MODEL TO PREDICT SURVIVAL IN PATIENTS WITH UNDIFFERENTIATED CARCINOMA OF UNKNOWN PRIMARY

A. van der Gaast[1,2], J. Verweij[1], A.S.Th Planting[1], W.C.J. Hop[3] and G. Stoter[1,2].

Department of [1]Medical Oncology, Rotterdam Cancer Institute.
Department of [2]Medical Oncology, University Hospital Rotterdam-Dijkzigt, The Netherlands.
Department of [3]Epidemiology & Biostatistics, Erasmus University, Rotterdam, The Netherlands.

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ABSTRACT

Purpose: We performed this study to identify prognostic factors in a subgroup of patients with carcinoma of unknown primary site treated with cisplatin combination chemotherapy.

Patients and Methods: Seventy-nine patients with poorly differentiated adenocarcinoma or undifferentiated carcinoma of unknown primary site were treated on two consecutive phase II chemotherapy protocols. The first protocol consisted of treatment with 3-week courses of cisplatin, etoposide and bleomycin (BEP). In the second protocol, cisplatin was administered weekly combined with oral administration of etoposide (DDP/-VP). To identify prognostic factors, univariate and multivariate analyses were conducted.

Results: In the univariate analysis, performance status, histology, liver or bone metastases, and serum levels of alkaline phosphatase and AST were significant variables to predict survival. In the multivariate analysis, performance status and alkaline phosphatase were the most important prognostic factors.

Conclusion: Good-prognosis patients had a performance score of 0 (World Health Organization [WHO]) and an alkaline phosphatase serum level less than 1.25 times the upper limit of normal (N). These patients had a median survival duration greater than 4 years. Intermediate-prognosis patients were characterized by either a performance status WHO $\geq$ 1 or an alkaline phosphatase level $\geq$ 1.25 N. These patients had a median survival duration of 10 months and a 4-year survival rate of only 15%. The poor-prognosis group had both a WHO performance status $\geq$ 1 and an alkaline phosphatase level $\geq$ 1.25 N. These patients had a median survival duration of only 4 months and none survived beyond 14 months. Treatment strategies for these three groups are discussed. It is suggested that this prognostic model be validated in other patient series.

INTRODUCTION

Many chemotherapy trials have been performed in patients with carcinoma of unknown primary site. Low response rates and brief median survival times were common in most series (1). A higher response rate to systemic treatment may be obtained in patients with favorable prognostic characteristics.

The identification of more responsive subgroups of patients is usually based on features such as age, performance status, sites of metastases, and histology. With regard to patients with tumors of unknown origin, well recognized subgroups are: (1) women with axillary lymph node metastases only; (2) women with peritoneal carcinomatosis only; (3) patients with poorly differentiated carcinoma with neuro-endocrine characteristics; (4) patients with undifferentiated carcinoma and poorly differentiated adenocarcinoma (2).

The latter subgroup was first described in 1979 and was named the atypical teratoma syndrome or the unrecognized extragonadal germ cell cancer syndrome, based on the contention that some of these patients had tumors of the germ cell lineage (3,4). In the early 1980s, the clinical definition of this subgroup of patients included the following criteria: (1) a histology of poorly differentiated adenocarcinoma or undifferentiated carcinoma of unknown primary site and, (2) one or more features of a treatable syndrome (5). These features consisted of the following: (1) age less than 50 years; (2) tumor primarily located in the midline; (3) elevated serum levels of human chorionic gonadotrophin (HCG) or alphafetoprotein (AFP); (4) clinical evidence of rapid tumor growth; or (5) tumor responsive to previously administered radiotherapy or chemotherapy.

After 1982, Hainsworth et al (6) included all patients with a histology of poorly differentiated adenocarcinoma or undifferentiated carcinoma of unknown primary in their treatment protocols and required no additional clinical features. In their largest reported series of 220 patients, treated with

cisplatin combination chemotherapy, an overall response rate of 62% was reported with an 10-year actuarial survival rate of 16%.

From 1988 onward, we have consistently treated patients with undifferentiated carcinoma and poorly differentiated adenocarcinoma of unknown primary site with cisplatin combination chemotherapy. Here, we report an analysis of putative prognostic variables in a cohort of 79 consecutive patients.

PATIENTS AND METHODS

Patients were entered onto two consecutive phase II chemotherapy protocols. The first protocol comprised treatment with cisplatin 20 mg/m² intravenously (IV) on days 1 to 5, etoposide 120 mg/m² IV on days 1, 3, and 5, and bleomycin 30 mg IV on days 2, 9, and 16 (BEP). On the second protocol, cisplatin 70 mg/m² IV was given on days 1, 8, 15, 29, 36, and 43, combined with etoposide 50 mg orally on days 1 to 15 and 29 to 43, followed by etoposide 50 mg/m² orally daily for 21 days every 4 weeks, for a maximum of four courses (DDP/VP). Tumor response was evaluated by standard World Health Organization (WHO) criteria (7) and response was evaluated after 2 cycles, ie, 6 or 8 weeks of treatment, respectively.

Patients were considered to have a carcinoma of unknown primary site if no primary tumor site could be identified after a thorough history and physical examination, chest x-ray, routine blood morphology and chemistry tests, mammography in women, visualization of the female pelvic organs, and determination of tumor markers such as prostate-specific antigen (PSA), HCG and AFP. An abdominal computed tomographic scan or ultrasound examination was performed in 97% of the patients. The histological specimens of patients referred from other centers were centrally reviewed. Immunohistochemical studies were performed on the tissue specimens of all patients, with the exception of those who had poorly differentiated adenocarcinomas confirmed by the presence of gland forma-

tion and mucin production. For the immunohistochemical studies, a panel of antibodies was used, which included antibodies against cytokeratins, vimentin and common leucocyte antigen (LCA). When necessary, this panel was extended by using more tumor-associated antibodies. Electron microscopy was performed on tumors with an inconclusive immunohistochemical pattern.

Most patients had one or more of the following clinical features: (1) age less than 50 years; (2) tumor located predominantly in a midline distribution (mediastinum or retroperitoneum), multiple pulmonary nodules, or lymphadenopathy; and (3) clinical evidence of rapid tumor growth. Patients with elevated serum levels of HCG or AFP were not eligible for this study. None of the patients had received prior chemotherapy.

Statistical methods

The following prognostic factors were analyzed: sex, age, performance status, histology, chemotherapy regimen, lung metastases, liver metastases, bone metastases, lactate dehydrogenase (LDH) level, alkaline phosphatase level, AST level, number of metastatic sites, and lymph node metastases as the predominant tumor site.

The significance of observed difference in proportions was tested using the Chi-square test. Survival was recorded from the start of chemotherapy to the date of death or last follow-up evaluation, and survival curves were calculated according to the method of Kaplan and Meier (8). Univariate survival analysis was performed with the log-rank test (9). Cox's regression was used to evaluate simultaneously the prognostic factors that were univariately significantly related to prognosis (10). $P \leq 0.05$ (two-sided) was considered the limit of significance.

Table 1. Patient characteristics

Characteristic		No. of patients	%
Total patients		77	
Sex	Female	48	62
	Male	29	38
Age, years			
	Median	52	
	Range	24 - 70	
WHO performance status			
	0	23	30
	1	39	51
	≥ 2	15	19
Histology			
	Poorly differentiated adenocarcinoma	43	56
	Undifferentiated carcinoma	34	44
Prior radiotherapy		10	13
Sites of disease			
	Liver	20	26
	Lung	13	17
	Bone	16	21
	Lymph nodes	54	70
	Mediastinal	30	39
	Retroperitoneal	16	21
	Other lymph nodes	33	43
	Skin metastases	10	13
	Miscellaneous	21	27
Regimen	BEP	59	77
	DDP/VP	18	23
Response	Complete	9	12
	Partial	23	30
	Stable disease	28	36
	Progressive disease	16	21
	Not assessable	1	

RESULTS

Of 79 treated patients, two were excluded because additional immunohistochemical examinations in retrospect showed that the correct diagnoses were malignant lymphoma and neuroblastoma, respectively.

Characteristics of the remaining 77 patients are listed in Table 1. Fifty-nine patients were treated with BEP and 18 with DDP/VP.

Nine patients (12%) achieved a complete response, with a median duration of 22+ months (range, 4 to 56+ months). Characteristics of complete responders are listed in Table 2. Of note, five complete responders had lymph node metastases only. Six complete responders are still disease-free. Twenty-three patients achieved a partial response with a median duration of 5 months (range, 3 to 21+ months). Twenty-eight patients had stable disease for a median duration of 4 months (range, 2 to 14 months) and 16 had progressive disease. One patient was not assessable for response and died after 6 months. Overall survival is presented in Fig 1, which shows a median survival of 8 months for the entire group of patients.

In the univariate analysis, significant variables to predict survival were performance status, histology, liver or bone metastases, and the serum levels of alkaline phosphatase and AST. Age, sex, lung metastases, lymph node metastases, serum levels of LDH, number of metastatic sites, and lymph node metastases as the predominant tumor site were not significantly related with survival. The results of the univariate analysis are listed in Table 3.

Table 2.

Characteristics of Complete Responders

Age (years)/Sex		Histology	Sites of disease	Survival (months)	Relapse
51	female	undiff. carcinoma	supraclavicular, mediastinal retroperitoneal lymph nodes, skin and liver metastases	8	brain
24	male	undiff. carcinoma	liver metastases, bone metastases	14	initial sites
54	male	undiff. carcinoma	inguinal lymph nodes	10+	no
58	male	undiff. carcinoma	mass 6 x 7 cm between bladder and rectum, iliacal lymph nodes	24+	no
37	male	undiff. carcinoma	mediastinal lymph nodes	23+	no
35	female	poorly diff. adenocarcinoma	liver metastases, inguinal, retroperitoneal lymph nodes	59+	no
58	male	undiff. carcinoma	supraclavicular lymph nodes mediastinal lymph nodes	31	brain
65	male	undiff. carcinoma	retroperitoneal lymph nodes	46+	no
46	male	poorly diff. adenocarcinoma	retroperitoneal lymph nodes	56+	no

Table 3.
Univariate prognostic analyses with survival as the endpoint

Variable	Median survival (months)	Significance
Histology		
adeno (n = 43)	6	p = 0.012
undiff ca (n = 34)	9	
Sex		
male (n = 48)	8	p = 0.49
female (n = 29)	6	
Age		
≤ 50 years (n = 35)	7	p = 0.83
> 50 years (n = 42)	7	
Performance (WHO)		
0 (n = 23)	9	p = 0.002
≥ 1 (n = 54)	3	
Regimen		
BEP (n = 59)	6	p = 0.09
DDP/VP (n = 18)	8	
Lung metastases		
Yes (n = 13)	6	p = 0.94
No (n = 64)	7	
Liver metastases		
Yes (n = 20)	4	p = 0.03
No (n = 57)	8	
Lymph node metastases		
Yes (n = 53)	8	p = 0.40
No (n = 24)	6	
Bone metastases		
Yes (n = 16)	4	p = 0.04
No (n = 61)	8	
Liver or bone metastases		
Yes (n = 30)	5	p = 0.005
No (n = 47)	9	

Number of metastatic sites			
1 site		13	
2 sites		7	p = 0.11
≥ 2 sites		6	
Predominant tumor site			
Lymph node metastases	(n = 28)	9	
Other	(n = 49)	6	p = 0.25
LDH (lactate dehydrogenase)			
< 1.25 N	(n = 52)	8	p = 0.22
≥ 1.25 N	(n = 25)	5	
Alkaline phosphatase			
< 1.25 N	(n = 40)	10	p < 0.001
≥ 1.25 N	(n = 37)	5	
SGOT			
< 1.25 N	(n = 58)	8	p = 0.03
≥ 1.25 N	(n = 19)	5	

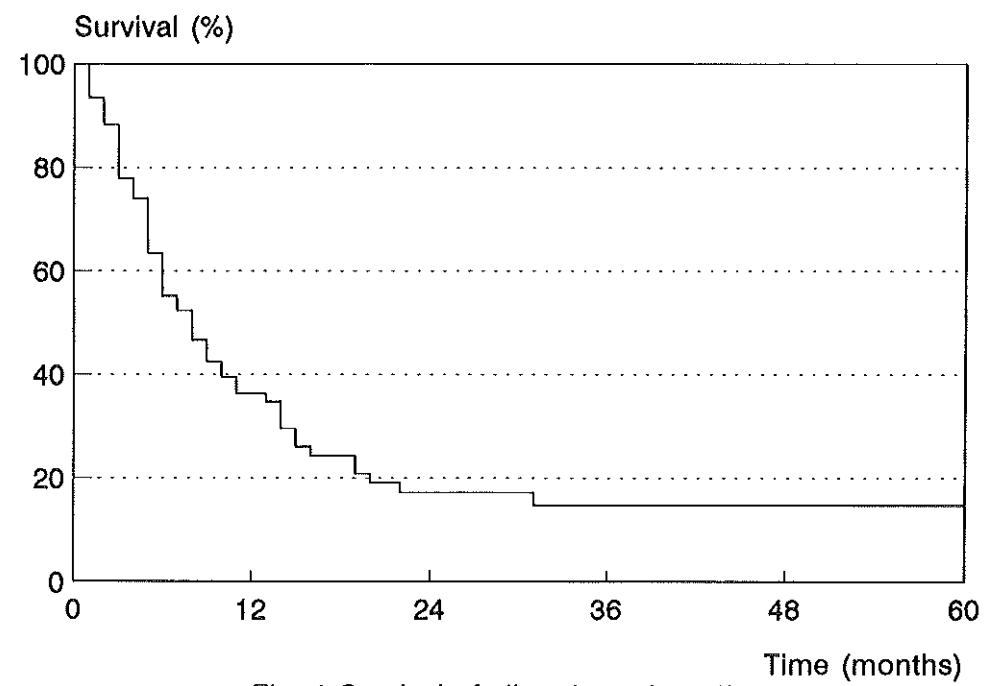


Fig. 1 Survival of all patients (n=71)

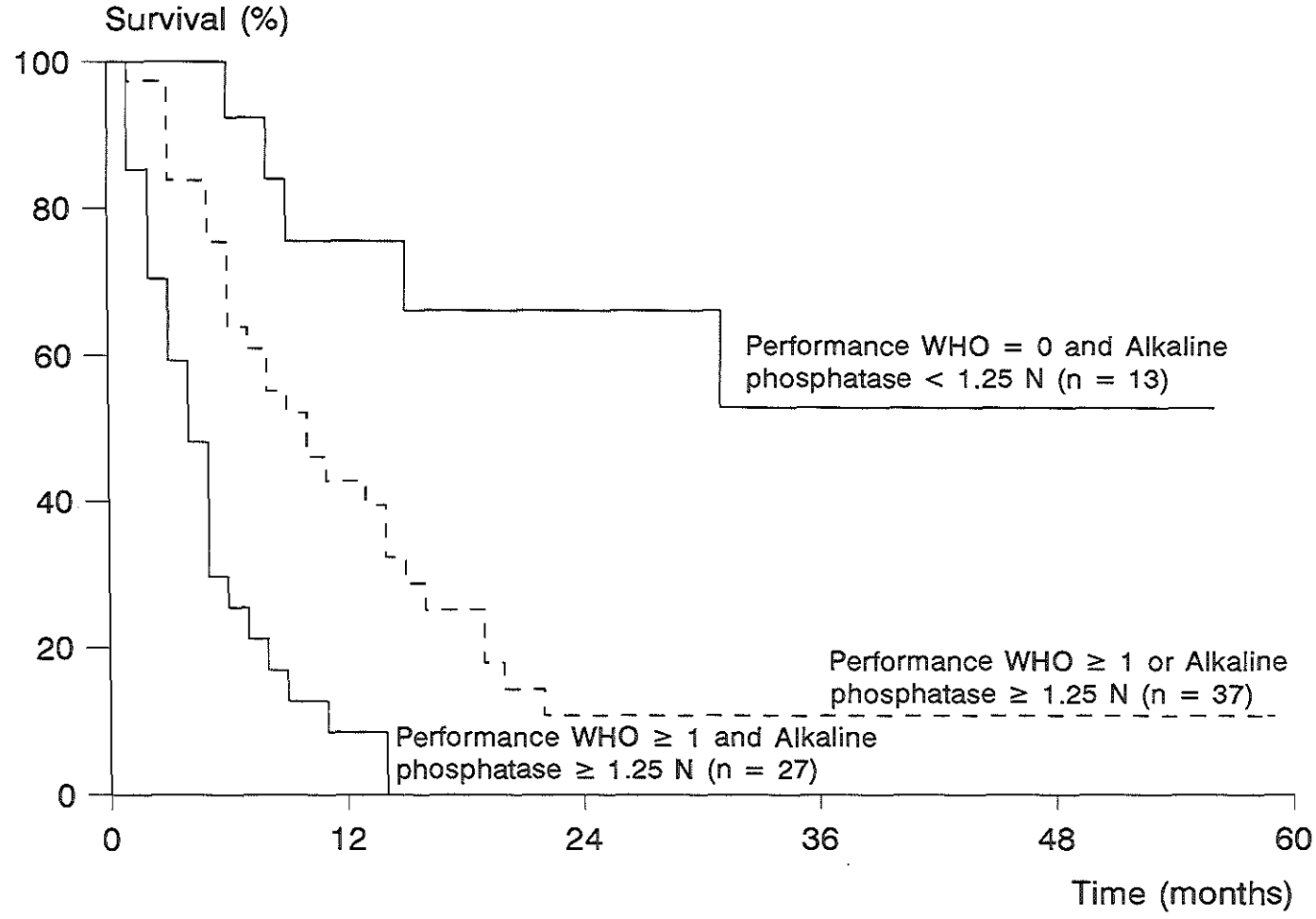
Multivariate analysis (Table 4) showed that performance status and alkaline phosphatase level were the most important prognostic factors and had about equal weight. Alkaline phosphatase was strongly correlated with the presence of bone metastases and ($P < .001$) and liver metastases ($P = .015$). The survival curves according to performance score and alkaline phosphatase are shown in Fig 2. The median survival duration of the 13 patients with a good performance status and a normal serum alkaline phosphatase level has not been reached after a follow-up duration of more than 4 years. The median survival of patients with one or two unfavorable prognostic factors are 10 and 4 months, respectively. The response rate in the group of patients with a WHO performance score of 0 and alkaline phosphatase level ≤ 1.25 times the upper limit of normal (N) was 69%. Patients with one or two unfavorable prognostic factors had response rates of 46% and 26%, respectively ($P = 0.25$).

Table 4.
Results of Cox's Multivariate Regression Analysis
With Survival as the End Point

Variable	Relative Death Rate	P
WHO performance status		
0	1	
≥ 1	3.3	< 0.001
Alkaline phosphatase		
< 1.25 N	1	
≥ 1.25 N	3.2	< 0.001

Figure 2

Survival according to performance status and alkaline phosphatase



DISCUSSION

Before the era of chemotherapy, patients with carcinoma of unknown primary site were treated, if at all, with surgery and/or radiotherapy. The identification of a primary tumor site had no high priority, since treatment with curative intent was only possible if the disease was confined to a single site. With the more widespread use of chemotherapy and the introduction of cisplatin, this issue gained interest because it was recognized that patients with extragonadal germ cell cancer could be cured, even when they had widespread disease.

The Vanderbilt group has reported the largest published experience in treating patients with undifferentiated carcinoma or poorly differentiated adenocarcinoma of unknown primary site with cisplatin-containing chemotherapy. This group reported a high response rate of 62% and a 10-year survival rate of 16% (6,11).

From 1988 onward, we have prospectively treated all patients with undifferentiated carcinomas and poorly differentiated adenocarcinomas of unknown primary site with cisplatin combination therapy. Thirty-two of 77 patients (42%) achieved a response, and it is estimated that the 5-year survival is 15% (Fig 1).

A multivariate analysis of prognostic factors showed that performance status and serum alkaline phosphatase level were the most important prognostic indicators. Clinically, it is understandable why serum alkaline phosphatase is such an important prognostic factor, because this variable is strongly correlated with liver and/or bone metastases. Based on performance status and alkaline phosphatase level, our series contained 13-good prognosis patients, 37 intermediate-prognosis patients, and 27 poor-prognosis patients. As shown in Fig 2, there is a large difference in the median and overall survival between these patient groups. The 2-year survival rates are 66%, 11%, and 0% for patients in the good-, intermediate-, and

poor-prognosis categories, respectively.

Our study and the Vanderbilt study (6) are the only reports in which a substantial number of patients with undifferentiated carcinomas of unknown primary site have been prospectively treated with cisplatin combination chemotherapy.

The overall and complete response rates of 42% and 12%, respectively, in our study are lower than the 62% overall and 26% complete response rates observed in the Vanderbilt study. The treatment regimens in both studies are similar, so the most likely explanation for the discrepant treatment results may be found in patient selection. We included more patients with liver and/or bone metastases. Our univariate prognostic factor analysis showed that these variables are associated with poor survival. To date, only one of our 20 patients with liver metastases was a long-term survivor, and none of the 16 patients with bone metastases survived beyond 18 months.

The median age in our study was 52 years, as opposed to 39 years in the Vanderbilt study. However, in contrast to the Vanderbilt study, age was not a significant prognostic factor in our study.

Besides younger age, predominant tumor location in the retroperitoneum or peripheral lymph nodes, tumor limited to one or two metastatic sites, and a negative smoking history were favorable prognostic factors in the Vanderbilt study. In our study, lymph node metastases as predominant tumor site and number of metastatic sites were not significant prognostic factors.

Despite the observed differences in patient selection and response rate, it is apparent that there are many similarities between both studies. In both studies only patients with an undifferentiated histology were included and all were prospectively treated with cisplatin combination therapy. In addition, the majority of patients had clinical evidence of rapidly progressive disease. Of note in both studies a small fraction of approximately 15% of the patients achieved long-term survival and may be cured.

Consequently, important questions to be answered are as follows: (1) should we treat all patients with undifferentiated carcinomas and poorly differentiated adenocarcinomas of unknown primary site with cisplatin combination chemotherapy?, and (2) what is the benefit of this treatment and for whom?

To answer these questions, it is essential to acquire more insight in the biology of these tumors and to define more precisely and uniformly which patients will obtain optimal results from systemic therapy. It is unlikely that we will be able to delineate favorable subgroups by the use of additional clinical features. However, the use of biomarkers and cytogenetic analyses may provide a better insight into the heterogeneity of these patients (12-13).

Until a uniform and independently validated prognostic model can be presented, some guidelines for treatment strategies based on the results of our study can be proposed.

Patients with a WHO performance status of 0 and normal serum alkaline phosphatase levels should always be treated with cisplatin combination chemotherapy, since the median survival is not reached after 4 years and cure may be possible.

Patients with one unfavorable prognostic factor (intermediate-prognosis group) have a low probability of long-term survival. However, the median survival of 10 months indicates that these patients may benefit in terms of palliation.

The third group of patients with two unfavorable characteristics should not be offered chemotherapy routinely, since their median survival is only 4 months and none of these patients survived beyond 14 months.

Finally, we suggest that our prognostic model be validated in other existing patient series.

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CHAPTER 7

CONCLUSIONS AND PERSPECTIVES

About 2% of all patients who present with cancer are diagnosed to have a carcinoma of unknown primary site. Despite the availability of newer diagnostic techniques such as computed tomography and magnetic resonance imaging, immunohistochemistry, and serum tumor markers, the incidence only slightly decreases (1).

Although the behavior of this disease entity, i.e. unknown primary, is aggressive with rapid proliferation of metastatic lesions these tumors are not very chemosensitive (2). In fact, for the majority of patients, especially those with well- and moderately differentiated adenocarcinomas, there is no available standard therapy. This situation can be explained by the fact that postmortem examinations reveals the pancreas, lung and colorectum as the primary tumor site in the majority of cases. Effective treatment for patients with metastatic adenocarcinoma from these organ is not yet available.

More encouraging is the situation for patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas. In these patients high response rates are obtained with cisplatin based chemotherapy and a small proportion of patients may obtain durable responses. However, even in this subgroup of patients it remains difficult to identify precisely those who benefit from treatment. In a prognostic factor analysis performed in the Vanderbilt series of patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas treated with cisplatin containing chemotherapy, age below 50, midline presentation, a limited number of metastatic sites and a negative smoking history were favorable prognostic factors (3). In our own series, as reported in chapter 6, multivariate analysis showed that patients with a WHO perfor-

mance status = 0 and normal levels of serum alkaline phosphatase have the best survival following chemotherapy.

In many solid tumors it is presently possible to detect genetic changes and the identification of specific cytogenetic lesions may have prognostic significance (4,5). Motzer et al. performed cytogenetic analysis on the tumor samples of 40 patients with poorly differentiated carcinomas of unknown primary site. Twelve patients had abnormalities of chromosome 12, i(12p), a deletion on the long arm of chromosome 12 or multiple copies of 12p; abnormalities also found in the majority of patients with germ cell tumors. Seventy-five percent of patients with chromosome 12 abnormalities responded to cisplatin containing chemotherapy as compared with an 18% response rate in the remaining patients (6). These findings suggest that by molecular diagnostic techniques additional prognostic factors may be identified.

In conclusion, until better therapies become available, the only way to improve treatment results in patients with metastatic disease of unknown primaries, is to utilize a combination of classical and molecular prognostic factors which will enable us to select with higher accuracy patients who will benefit from treatment.

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SUMMARY

Chapter 1 is a review of the literature of patients with carcinomas of unknown primary site.

A phase II study with the combination 5-fluorouracil, doxorubicin, and mitomycin-C (FAM) for patients with adenocarcinomas of unknown primary site is reported in chapter 2. Three out of 22 (14%) evaluable patients achieved a partial response. FAM cannot recommended for routine use in patients with adenocarcinomas of unknown primary.

In chapter 3 the results are reported of a phase-II study with etoposide 50 mg/m² orally for 3 weeks every 4 weeks administered to patients with well- and moderately differentiated adenocarcinomas of unknown primary site. Two out of 24 (8%) evaluable patients obtained a partial response. It is concluded that etoposide given in this dosage and schedule has only limited activity in this group of patients.

The results of a phase-II study with combination chemotherapy with cisplatin, etoposide, and bleomycin in patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas are reported in chapter 4. More than half of the patients responded to this treatment and some complete responders had durable responses.

The value of immunohistochemistry in patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas is described in chapter 5. In a number of patients the diagnosis had to be changed or could be refined. Immunohistochemistry should be routinely performed in patients with undifferentiated carcinomas of unknown primary site.

In chapter 6 a prognostic model is presented based on the results of a multivariate analysis performed in 79 patients with poorly differentiated adenocarcinomas or undifferentiated carcinomas, all treated with cisplatin containing combination chemotherapy. Patients with a performance status WHO = 0 and normal levels of serum alkaline phosphatase had the best prognosis.

In chapter 7 it is concluded that treatment in patients with carcinomas of unknown primary should be guided by prognostic factors based on both patients and tumor characteristics.

SAMENVATTING

In hoofdstuk 1 wordt een kort literatuur overzicht gegeven over patiënten met een carcinoom van onbekende origine.

In hoofdstuk 2 worden de resultaten beschreven van een fase-II onderzoek met de combinatie van 5-fluorouracil, adriamycine en mitomycine-C bij 23 patiënten met een adenocarcinoom van onbekende origine. Een partiële response werd bij slechts 3 van de 22 evalueerbare patiënten waargenomen. De conclusie was dat deze behandeling niet kan worden aanbevolen als standaard behandeling voor deze groep van patiënten.

In hoofdstuk 3 worden de resultaten beschreven van een fase-II onderzoek met een schema van oraal toegediend etoposide 50 mg/m² gedurende 3 weken per 4 weken bij patiënten met een goed of matig gedifferentieerd adenocarcinoom van onbekende origine. Twee van de 24 evalueerbare patiënten behaalden een partiële response. Geconcludeerd werd dat etoposide toegediend in deze dosering en in dit schema slechts een geringe activiteit heeft in deze groep van patiënten.

In hoofdstuk 4 worden de resultaten beschreven van cisplatina bevattende chemotherapie bij patiënten met een slecht gedifferentieerd adenocarcinoom of een ongedifferentieerd carcinoom van onbekende origine. Meer dan de helft van de patiënten reageerden gunstig op deze behandeling. De response duur bij een aantal complete responders is lang en deze patiënten zijn mogelijk gecureerd.

De waarde van immunohistochemisch onderzoek wordt beschreven in hoofdstuk 5. Bij een aantal patiënten moest op grond van dit onderzoek de diagnose worden bijgesteld of kon de diagnose worden verfijnd. De conclusie was dat immunohistochemisch onderzoek routinematig dient te worden toegepast bij patiënten met een ongedifferentieerd carcinoom van onbekende origine.

In hoofdstuk 6 wordt een prognostisch model gepresenteerd gebaseerd op de resultaten van een multivariant analyse uitgevoerd in een groep van 79 patiënten met een slecht gedifferen-

tieerd adenocarcinoom of een ongedifferentieerd carcinoom van onbekende origine, allen behandeld met cisplatina bevattende chemotherapie. Met name patiënten met een "performance score" van WHO = 0 en een normaal serum alkalisch fosfatase vallen in de meest gunstige prognose groep.

In hoofdstuk 7 wordt gesteld dat bij gebrek aan effectievere behandelingen een verbetering van behandelingsresultaten alleen mogelijk is door een verfijning van prognostische factoren gebaseerd op patient en tumor karakteristieken.

NAWOORD

Een klinisch proefschrift komt slechts tot stand door samen te werken met anderen. Het zal duidelijk zijn dat ook de lijst van co-auteurs een beperkte afspiegeling is van het aantal mensen die hebben meegewerkt om de verschillende klinische studies tot een goed eind te brengen. Iedereen die op een of andere manier aan dit proefschrift heeft meegewerkt zeg ik hierbij hartelijk dank.

Prof. G. Stoter, beste Gerrit: Dank voor je helder inzicht en je gave mijn bij tijd en wijle krom taalgebruik weer recht te breien. Ik heb er spijt van dat we de promotiedatum niet eerder hebben gepland. Hierdoor is het niet meer mogelijk je "eerste" promovendus te zijn. Ik heb er geen spijt van dat ik een van je eerste adviezen, om de behandeling van patiënten met tumoren van onbekende origine maar samen te vatten op één A-4tje, niet heb opgevolgd.

Dr. J. Verweij, beste Jaap:. Jij bent degene die aan de bakermat van dit proefschrift stond en je bent ook degene die mij begeleidde bij mijn eerste schreden op het pad van het klinisch oncologisch onderzoek. Bedankt voor dit alles.

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In de eerste plaats gaat mijn dank uit naar het thuisfront, mijn vrouw en kinderen, voor de rust en liefde die zij mij geven, niet zozeer noodzakelijk voor de voltooiing van dit proefschrift maar wel voor mijn dagelijks functioneren.

CURRICULUM VITAE

Ate van der Gaast

20 juni 1956	: geboren te Hommerts.
1968 - 1974	: VWO, J. Bogerman College te Sneek.
1979	: Doctoraal Examen geneeskunde, Rijksuniversiteit Groningen.
1981	: Artsexamen, Rijksuniversiteit Groningen.
1981 - 1986	: Specialisatie Inwendige geneeskunde, het St. Geertruiden Ziekenhuis te Deventer, opleider J.B. Scholten.
1986 - 1988	: als internist werkzaam op de afdeling medische oncologie van de Dr. Daniel den Hoed kliniek te Rotterdam.
1988 - heden	: verbonden als internist aan de afdeling medische oncologie van het Academisch Ziekenhuis Rotterdam-Dijkzigt

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