의학석사 학위논문

원발부위 미상 암으로 진단 받은 환자의 특성과 치료 성적을 보고자 하는 코호트 연구

Real-world data analysis of cancer of unknown primary

울산대학교 대학원

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2021 년 2 월

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Abstract

Background: Cancer of Unknown Primary (CUP) is a heterogeneous malignancy group, without a recognizable primary site of the tumor through standard workup. Survival outcome is generally poor, with median overall survival (OS) of 6 ~ 12 months. Standard treatment of CUP was not established yet, empirical cytotoxic chemotherapy is widely used. To determine treatment patterns and survival outcomes in patients with cancer of unknown primary (CUP) in real-world settings, we conducted a retrospective study of patients who were diagnosed with CUP and treated. **Materials and Methods**: From January 2009 to December 2019, 218 patients who were diagnosed with CUP at Asan Medical Center were identified. We retrospectively analyzed baseline patient characteristics, treatment patterns and survival outcomes. For some patients, next-generation sequencing (NGS) results were analyzed. **Results**: Median patient age was 62 years (range 19-91); 62.3% were male. Eighty-five percent of patients had initially disseminated metastatic disease. Among 22 patients (10.09%) who underwent NGS, an actionable clinical alteration was seen in 3 patients. Most patients (n=132, 60.3%) underwent empirical cytotoxic chemotherapy. Two patients received targeted treatment based on NGS results. Forty-six patients underwent surgery, and 66 patients received radiation therapy. Median overall survival for all patients was 8.25 months (95% Confidence interval [CI] 6.18-11.44). Median progression-free survival for patients treated with at least one line of chemotherapy was 4.37 months (95% CI 3.35-5.33). In multivariate Cox regression analysis, the small number of metastatic sites (≤ 2), better Eastern Cooperative Oncology Group performance status (ECOG PS, 0 or 1) and localized disease were associated with better survival outcome. **Conclusion**: Standard treatment in a real-world setting led to poor prognosis in CUP. Patients with localized disease received local treatment or those with fewer metastasis and better PS treated with multiple lines of chemotherapy showed better survival. Novel targeted therapy based on NGS results is expected to improve survival outcomes, warranting further investigation.

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Introduction

Cancer of unknown primary (CUP) is a heterogeneous malignant condition for which the primary site of origin has not been identified by general diagnostic evaluation[1]. It accounts for 3-5% of all human malignancies, but recently, this number has decreased to 1-2% owing to advances in diagnostic methods including molecular profiling[2]. Most patients with CUP have been included in unfavorable subsets and shown poor prognosis, with a median overall survival (OS) of 6 months[1]. Because of the heterogeneity of CUP, there has been no consensus on standard treatment of CUP was not established yet. Generally, empirical cytotoxic chemotherapy and palliative radiotherapy have been widely used for CUP patients.

Recently, gene expression profiling (GEP) and next generation sequencing (NGS) have gained attention as tools to detect the primary site and to study the molecular features of CUP[3-6]. Moreover, research on site-specific therapy and targeted therapy based on GEP, and NGS results in CUP patients has been increasing[7-10]. Some prospective studies have demonstrated better survival outcomes after tailored therapy than after empirical cytotoxic chemotherapy[7 8], but some randomized trials reported no significant differences in survival outcomes between the two groups[9 10]. This suggests that there is some discrepancy between studies in terms of the efficacy of the novel targeted therapy for CUP[11].

Despite recent advances in diagnostic techniques and treatment strategies, current knowledge of the characteristics of CUP and treatment patterns in real-world settings are not well known. The clinical utility of tailored therapy is also not yet established. However, there are a few studies on how novel targeted therapy is actually being applied in clinical practice. For this reason, we conducted a retrospective study of patients who were diagnosed with CUP and underwent treatment at Asan Medical Center, Seoul, Korea, to determine clinical and molecular characteristics, treatment patterns, survival outcomes, and efficacy of NGS and targeted therapy in patients with CUP in real-world settings.

Materials and Methods

Patients

Between January 2009 and December 2019, patients who were diagnosed with CUP in our institution's cancer registry were identified and enrolled. CUP was defined according to the initial ICD code, which was an "unknown primary site" (ICD-0-3 code 80.9). Exclusion criteria were as follows: patients whose primary site was identified later through further imaging studies and/or

histologic diagnosis with additional immunohistochemical staining. We reviewed the medical records of all included patients to collect data on demographic and clinical characteristics including age, sex, Eastern Cooperative Oncology Group performance status (ECOG-PS), histopathological diagnosis, number and location of metastases, disease extent, treatment strategy, chemotherapy regimen, response to chemotherapy, and survival outcome. Disease extent was classified as localized disease and disseminated disease, where localized disease was defined as a single lymph node region or single site. All non-localized cases were classified as disseminated disease. Additionally, we identified patients who underwent NGS and analyzed their NGS results.

Statistical analysis

OS was defined from the date of pathological diagnosis to the date of due to any cause. Progression-free survival (PFS) was calculated from the date of chemotherapy initiation to the date of documented progression or death. If the patient was alive on the date of the last outpatient visit and lost to follow-up, then we censored them at the date of the last outpatient visit date. We used the Kaplan-Meier method to estimate OS and PFS and used the log-rank method to compare OS and PFS between subgroups. To determine the prognostic value of variables, multivariate Cox regression analysis was performed. $P < 0.05$ was considered as statistically significant. All statistical analyses were conducted by using R statistical software (version 3.6.3)[12]

Ethical approval

This retrospective study was reviewed and approved by The Institutional Review Board of Asan Medical Center (Seoul, South Korea).

Results

Patient characteristics

Between January 2009 and December 2019, a total of 218 patients with CUP were identified in the Asan Medical Center cancer registry. During diagnostic work-up, medical history, physical examination, baseline blood and biochemistry analyses, imaging studies including chest and abdomen-pelvis computed-tomography (CT), and biopsy were performed for all patients. Additionally, 94% (n = 206) of patients underwent positron emission tomography (PET)/CT as a part

of a the diagnostic workup. **Table 1** shows the data of patient characteristics. Median patient age was 62 years (range 19-91) and males accounted for 62.3%. Furthermore, 142 patients (65.1%) had initial ECOG PS 0 or 1, and 76 patients had 2 or more than 2 of ECOG PS. Approximately 85% of patients had disseminated disease, and the median number of metastatic sites was 2 (range 1-8). In patients with localized disease, the most metastatic sites were lymph nodes (n = 17, 51.5%), followed by intra-abdominal, and pelvic regions. (n = 5, 15.2%). In disseminated disease, the most metastatic sites were the bones (46.4%), liver (40%), lung (27%), peritoneum (18.9%), and pleural effusion (10.2%). Approximately 80% of patients showed lymph node metastases (n = 148). According to the classification of histologic subtypes, carcinoma not otherwise specified (NOS) including poorly differentiated adenocarcinoma accounted for more than half of the patients (n = 122, 55.9%). Squamous cell carcinoma and neuroendocrine tumor accounted for 16% and 13%, respectively.

Molecular characteristics of patients with CUP

Twenty-two patients (10.1%) underwent NGS. Level 2 or 3 alteration was seen in 13 patients, and clinically actionable alteration was seen in 3 patients[13]. Details of NGS results were presented in **Table 2.**

Treatment patterns

A total of 58 patients (26%) did not receive treatment in our center. Among them, 20 were transferred to other hospitals after diagnosis, 11 refused to undergo treatment, 23 were unable to receive treatment due to poor PS, and 4 were lost to follow-up after the initial diagnostic workup (**Table 3**).

Among 218 patients, 132 (60.7%) received first-line chemotherapy. Among them, 70 were treated with second-line chemotherapy, and 28 and 6 received third- and fourth-line therapy, respectively. Details of the first- and second-line chemotherapy regimens are shown in **Table 4**. The most common regimen used as first-line chemotherapy was FP (5-fluorouracil [FU] and cisplatin, n = 74), followed by etoposide and cisplatin (EP, n = 20), paclitaxel and carboplatin (PC, n = 13). Responses to first-line chemotherapy were as follows: 2 cases of complete remission (CR), 18 of partial response (PR), 28 of stable disease (SD), 53 of progressive disease (PD), and 31 of non-evaluable. The overall response rate (ORR) was 15%. PFS after first-line chemotherapy was 4.4 months (95% confidential interval [CI]) 3.4-5.3, **Figure 1**). The most common reason for the discontinuation of first-line chemotherapy was disease progression (27.3%).

Table 1 Patients characteristics

Characteristics	**n** = 218	**%**
Gender		
Male	136	62.39
Female	82	37.44
Age		
≥ 60	93	42.47
< 60	125	57.34
ECOG Performance status		
0, 1	142	65.14
≥ 2	76	34.7
NGS		
Done	22	10.09
not done	196	89.49
Disease extent		
Localized disease	33	15.13
Bone	2	6.1
Extremity	1	3.0
Head & neck lesion	1	3.0
Intestine	1	3.0
Intra-abdominal, pelvis mass	5	15.2
Liver	2	6.1
Lymph nodes*	17	51.5
Mediastinal mass	2	6.1
Peritoneum carcinomatosis alone	2	6.1
Disseminated disease	185	84.86
Adrenal gland	15	8.1
Bone	86	46.4
Brain	7	3.7
Head and neck lesion	7	3.7
Intra-abdominal organ except liver†	50	27

Table 1. (continued)

Disease extent		
Disseminated disease	**n**	%
Liver	74	40
Lung	50	27
Lymph nodes	148	80
Muscle	5	2.7
Peritoneum	35	18.9
Pleural effusion	19	10.2
Skin	4	2.1

*includes cervical (10), Inguinal (5), hepatoduodenal (1) and axillary (1) lymph nodes.

†includes pelvic mass (8), pancreas (7), Gallbladder(7), Ovary (6), intestine(6), stomach (4), intra-abdominal mass (3), spleen(3), kidney & ureter(3), retroperitoneal mass(2), and bladder(1).

Abbreviations: ECOG, Eastern Cooperative Oncology group; NGS, Next generation sequencing

Table 2 Clinical and molecular characteristic of patients who underwent NGS

ID	Anatomical location	Histology	Gender	Age	Level 2 / 3 gene alteration	Amino acid
1	Lt paraaortic, aortocaval lymph node and Small bowel, mesentery area	AdenoCa	Male	66	**LMNA-NTRK1** PALB2 T993M (level 2)	TP53 V73Wfs*50 RNF43 G659Vfs*41, L311Sfs*108 POLE R266* Broad-segment copy number gain of 1q21.1-44 region SMAD4 K110Nfs*12
2	bone(skull, right 7th, left 8th ribs, T8, T10, T12, L4, right ischium, left pelvic bone)	Neuroendocrine Ca	Male	49		POLE T41M RB1 (K122E) AXIN1(S57L) PIK3CB (R321Q)
3	LN (left cervical, left supraclavicular, retroperitoneum, both iliac)	Squamous cell Ca	Female	73		PTEN truncating mutation (Q17*) IGF1R
4	Lt SCN, mediastinal LN, retroperitoneal LNs, pelvic nodes, bones	Squamous cell Ca	Female	45	**PIK3CA E545K**	AKT3_L313P, BAP1_S63C, NOTCH4_G1623R CREBBP_L74V SMARCA4_L1073Q

Table 2. (continued)

5	Peritoneal carcinomatosis	AdenoCa	Female	70	TP53 V73M BRCA2 E3167D	SMAD4 P356L
6	Left adrenal gland, LN(retroperitoneum, left supraclavicular)	AdenoCa	Female	50	**NRAS G12D** BRAF G466V	TP53 E68*, RNF43 R145* SMAD4 Q442*
7	Lung(RLL mass), LN(both lung), right kidney, proximal ureter	Ca NOS	Female	59		PTEN loss (chr 10) AKT2 gain
8	Bone, lung	Ca NOS	Male	61	RICTOR amplification	
9	LN(left supraclavicular, bilateral retrocrural, left gastric, common hepatic, hepatoduodenal ligament, mesenteric, retroperitoneal areas)	AdenoCa	Male	59	TP53 R175G CDK6 and CASD1 genes (7q21.12-21.2) CCND1 and FAM86C CDK4 or CDK6 PDGFRA I989L	
10	Intra-abdominal LN	AdenoCa	Female	52	SMAD4 R361C TP53 X126_splice	

Table 2. (continued)

11	Pelvic bone	AdenoCa	Female	70	KRAS G12S TP53 C238Y CDKN2A/2B(p16INK4A and p14ARF)	ERBB2-PPP1R1B
12	LN(para-aortic, aortocaval, Lt. common ilia LNs)	Ca NOS	Female	57	BRAF V600E TP53 V173M MYC amplification (21 copies)	Multiple segmental copy number gains and losses are detected.
13	Inguinal LN	Squamous cell Ca	Female	54	PIK3CA E545K	FBXW7 R465C XRCC2 R64* ARID2 N1778Ifs*13 RNF43 G659Vfs*41 SMARCA4 X473_splice ACVR2A
14	Perigastric LN	adenoCa	Male	75	ERBB2 S310F	SMARCA4 E1023*
15	Mediastinal LN	adenoCa	Female	61	BRAF G649A	ARID1A W1686*/D1850Gfs*4 CTNNB1 S45F NF1 R1362* FLT4 A1158V

Table 2. (continued)

16	Lung	AdenoCa	Female	72	TP53 R175H ARID2 alterations (K706*, T1540Nfs*4)	NF1 H1943Lfs*19 PTEN CDKN2A/2B
17	Mediastinal LN	Squamous cell Ca	Male	55	BRCA2 I1859Kfs*3 CREBBP Q955* TP53 alterations CCND1 amplification CNV Loss of 9p21.3 harboring CDKN2A/2B genes,	NOTCH1 E1827*
18	Bone	Ca NOS	Male	68		CDKN2A H66R mutation
19	Right external LN	Ca NOS	Male	73	TP53 X307_splice ATM associated rearrangement CCND1 (~7 copies)	

Table 2. (continued)

20	Hepatoduodenal LN	Ca NOS	Female	68	(L442_Q446del, Q754*) of ATM	Amplifications of 4q12 (PDGFRA, KIT, KDR; 17 copies) 12q13.3-15 (ERBB3, MYO1A, CDK4, MDM2; 9 copies) 19q12 (CCNE1, 7 copies) loss of 99p21.3 (CDKN2A/2B)
21	Cervical LN	Ca NOS	Male	56	TP53 C242R TSC N762S	Strong EGFR amplification (~ 30 copies)
22	Hepatoduodenal LN	Neoplasms NOS	Male	72	KRAS G12A TP53 R213*	

Abbreviations; AdenoCa, adenocarcinoma; Ca, carcinoma; NOS, not otherwise specified.

Table 3 Treatment patterns

Treatments	n = 218	%
Chemotherapy	132	60.5
Operation	46	21.1
For curative resection	13	
For palliative purpose	14	
Radiotherapy	66	30.2
Adjuvant RT/Definite RT*	19	
Palliative RT	47	
No treatment	58	26.6

Abbreviation; RT, radiotherapy.

*includes Concurrent chemo-radiotherapy(10)

Table 4 Chemotherapy regimen of 1st line and 2nd line in CUP patients.

Chemotherapy regimen		
1st line chemotherapy regimen	**n = 132**	
FP (5-FU, Cisplatin)	74	56.1
EP (Etoposide, Cisplatin)	20	15.2
PC (Paclitaxel, Carboplatin)	13	9.8
Clinical study	4	3.0
GP (Gemcitabine, Cisplatin)	3	2.3
VIP (Cisplatin, Etoposide and Ifosfamide)	3	2.3
EC (Etoposide, Carboplatin)	2	1.5
FEP (5-FU, Etoposide and Cisplatin)	2	1.5
FOLFIRI(5-FU, Leucovorin and Irinotecan)	1	0.8
FOLFOX(5-FU, Leucovorin and Oxaliplatin)	1	0.8
TP (Carboplatin, Cisplatin)	1	0.8
Others*	8	6.1
2nd line chemotherapy regimen	**n = 69**	
PC (Paclitaxel, Carboplatin)	12	17.4
GP (Gemcitabine, Cisplatin)	11	15.9
CAV (Cyclophosphamide, Doxorubicin and Vincristine)	9	13.0
FP(5-FU, Cisplatin)	7	10.1
CYVADIC (Cyclophosphamide, Vincristine, Doxorubin and Dacarbazine)	5	7.2
Paclitaxel	4	5.8
Docetaxel	3	4.3
FOLFOX(5-FU, Leucovorin and Oxaliplatin)	3	4.3
CAP (Cyclophosphamide, Doxorubicin and Cisplatin)	2	2.9
EP (Etoposide, Paclitaxel)	2	2.9
Pembrolizumab	1	1.4
Entrectinib	1	1.4
Others†	9	13.0

*includes Casodex/lucrin (1), CHOP(cyclophosphamide, doxorubicin, vincristine and prednisolone) (1), DFP(docetaxel, 5-FU and CDDP)(1), Gemcitabine(1), octreotide(2), and

CVD(Cyclophosphamide, vincristine and dacarbazine) (1).

†includes XELOX(Oxaliplatin, capecitabine) (1), Afinitor(1), gemcitabine(1), irinotecan(1), IP(1), VIP(Etoposide, ifosfamide, and cisplatin)(1), XP(capecitabine, cisplatin)+Herceptin(1), ICE(ifosfamide, carboplatin and etoposide) (1) and AP(doxorubicin, cisplatin) (1)

abbreviations; CUP, Carcinoma of unknown primary; FU, Fluorouracil.

The most common regimen used as second line chemotherapy was PC (n = 12), followed by GP (gemcitabine and carboplatin, n = 11), CAV (Cyclophosphamide, Doxorubicin, and Vincristine, n = 9) and FP (n = 7). The best response to second line chemotherapy was PR (8.5%), and 42.8% of patients showed PD. Median PFS after second line chemotherapy was 2.1 months (95% CI 1.94 - 3.95). Details of third line and fourth line chemotherapy regimens are summarized in Table S1.

Targeted therapy was provided to two patients based on NGS results. A patient who had NTRK fusion initially was treated for GC (gemcitabine and cisplatin), but it progressed after 3 months (5 cycles). He was treated with entrectinib as second-line therapy, which is an Food and Drug Administration (FDA) approved targeted therapy for solid tumor with NTRK fusion. He remained in a stable disease state for 9 months while taking entrectinib, but the disease progressed. Currently, he has participated in the clinical trial of immune checkpoint inhibitor (PDR-001) as third line therapy. Other patients who had AKT2 gain mutation were treated with ipatasertib, which is an FDA-approved targeted therapy as an AKT inhibitor. He was diagnosed with CUP with involvement of the liver, lung, right ureter and multiple lymph nodes (retroperitoneal, mediastinal, left supraclavicular) and therefore treated with ipatasertib after progression of PC and GP chemotherapy. However, he died due to liver failure, after 2 weeks of ipatasertib treatment.

Immunotherapy was provided to three patients. One patient with inguinal area lymphadenopathy and squamous cell carcinoma was treated with PC as first line chemotherapy. After 6 cycles of PC, he remained in the SD status for 6 months, during drug holiday. After that, disease progressed, and pembrolizumab was therefore administered as second line chemotherapy, based on microsatellite instability (MSI) high status as observed on immunohistochemistry. PFS was approximately 6 months during pembrolizumab therapy, and FP and re-do PC were administered as third- and fourth-line chemotherapy regimens, respectively. He died due to septic shock and pneumonia. Another patient had anterior mediastinal mass, and biopsy showed poorly differentiated adenocarcinoma. She was treated with VIP (etoposide, ifosfamide and cisplatin), AP (doxorubicin, cisplatin), IP (irinotecan, cisplatin), and palliative radiation therapy, but the disease was progressed. As fourth line chemotherapy, pembrolizumab was administered once, but she died within 2 weeks before disease evaluation. The last case is a patient who was treated with entrectinib, and currently, the patient is participating in a clinical trial of PDR-001.

Forty-six patients underwent operation. Surgery was performed for diagnosis in 17 patients, with curative intent in 13 patients, and for palliation in 14 patients. Surgeries for palliative purposes included the following: decompression operation due to spinal bone metastasis, bowel resection due to obstruction, internal fixation of humerus due to pathologic fracture related to bone metastasis,

abdominal tumor mass excision for pain control, and brain tumor resection.

A total of 66 patients received radiotherapy (RT). Among them, 19 received definite RT (including 10 patients who were underwent concurrent chemoradiotherapy (CCRT)) and 47 patients received palliative RT for pain control. Among 10 patients who were treated with CCRT, 5 were diagnosed with squamous cell carcinoma at the head and neck lesion, 1 with squamous cell carcinoma at mediastinum, 1 with squamous cell carcinoma in the lymph node on the inguinal lesion, and 3 with poorly differentiated carcinoma at the cervical lymph node region. Median OS for patients treated with CCRT was 51.7 months (95% CI, 40.4–Not reached [NR]).

Clinical Outcomes and Prognostic Factors

Median OS for all patients was 8.3 months (95% CI, 6.2-11.4) (**Figure 2**). Median OS was 13.3 months (95% CI, 9.00-18.5) for ECOG PS 0-1, and 3.9 months (95% CI, 2.7-6.0) for ECOG PS greater than 1 (**Figure 3a**). The OS according to disease extent is shown in Figure 3b. Median OS was 34.6 months (95% CI, 24.5–NR) and 6 months (95% CI, 4.7–8.3) for localized disease and disseminated disease, respectively**.** Figure 3c shows survival curves for patients classified by histology, and patients with squamous cell carcinoma showed better outcome than patients with other histologic types (median OS - 27.8 months, 95% CI, 13.4 - NR). Carcinoma NOS and poorly differentiated adenocarcinoma showed the worst outcome (median OS 4.7 months, 95% CI, 3.5-6.8). However, squamous cell carcinoma was not a significant prognostic factor in subgroup analysis according to disease extent (**Figure S1**), and median OS of patients who received only first-line chemotherapy and second-line chemotherapy was 4.7 months (95% CI, 3.1 - 8.4) and 9.6 months (95% CI, 8.3 - 16.3), respectively. Furthermore, median OS of patients treated with third-line chemotherapy and fourth line chemotherapy was 23 months (95% CI, 14.0 - NR), and 29.4 months (95% CI, 15.6 - NR), respectively (**Figure 3d**).

We performed univariate and multivariate Cox regression tests to determine prognostic factors related to survival outcomes in patients with CUP. In univariate analysis, age greater than 60 and sex were not associated with survival outcome. Otherwise, ECOG PS (hazard ratio [HR] 2.25, $p < 0.001$) and localized disease (HR 3.55, $p < 0.001$) were significantly related to better OS. In multivariate analysis, all two factors significantly associated with survival outcome in univariate analysis were significantly related to survival outcomes (**Table 4**)

Table 5 Univariate & multivariate Cox-regression analysis of potential prognostic factors of overall survival in patients with CUP.

	Univariate		Multivariate	
Factor	HR ratio (95% CI)	P value	HR ratio (95% CI)	P value
Gender([a]male)	0.87 (0.62-1.22)	0.447		
Age([a] age<60)	0.99 (0.71-1.37)	0.958		
ECOG PS([a] ECOG PS 0,1)	2.47 (1.76-3.48)	<0.001	2.25(1.59-3.17)	<0.001
Disease extent ([a] localized disease)	3.71(2.12-6.50).	<0.001	3.55(2.02-6.25)	<0.001

Abbreviations; CI, confidence interval; HR, hazard ratio, ECOG PS, Eastern Cooperative Oncology Group Performance Status

Figure 1 Progression-free survival for patients with CUP, who treated with Chemotherapy

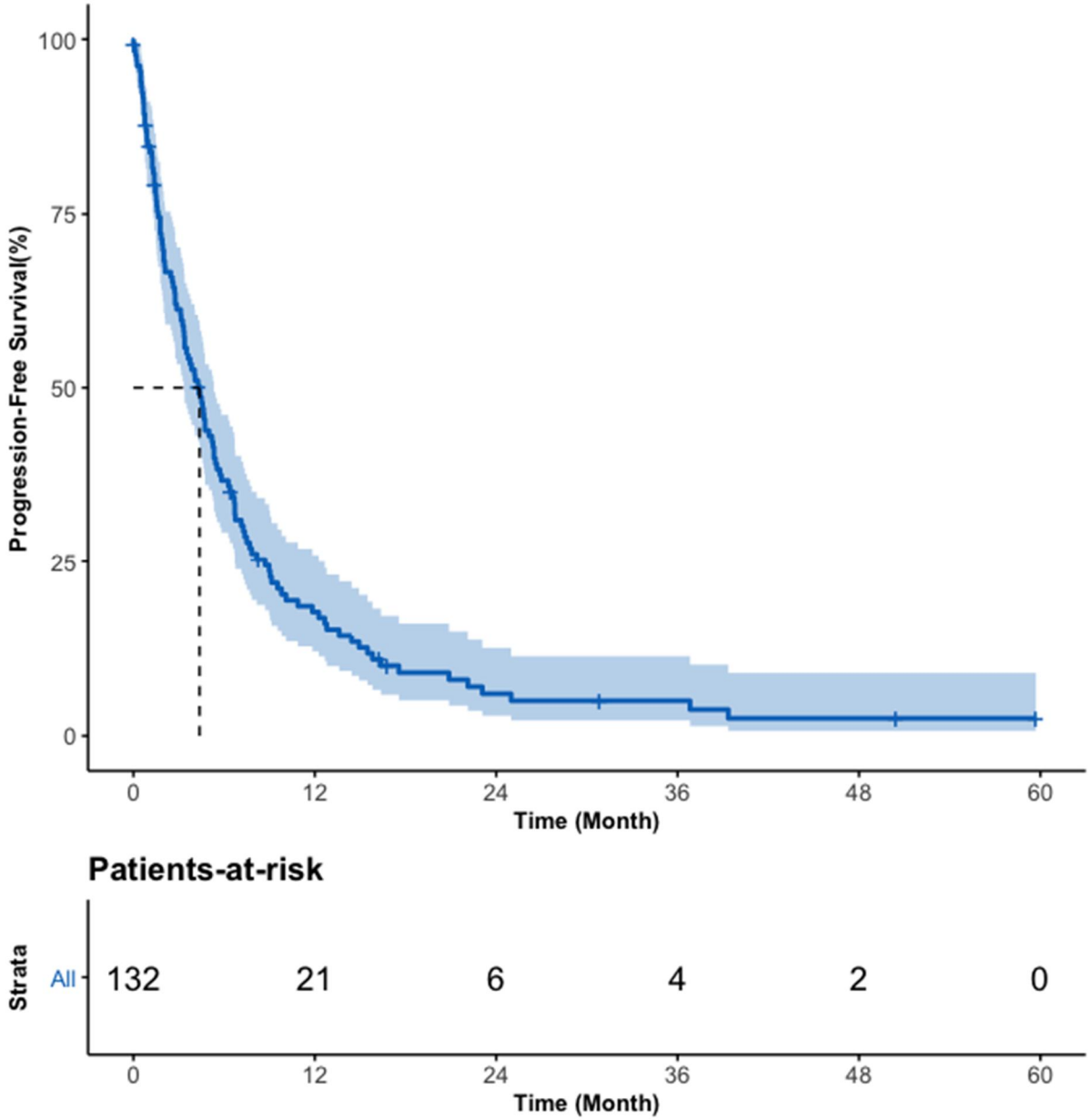


Figure 2 Overall survival for patients with CUP

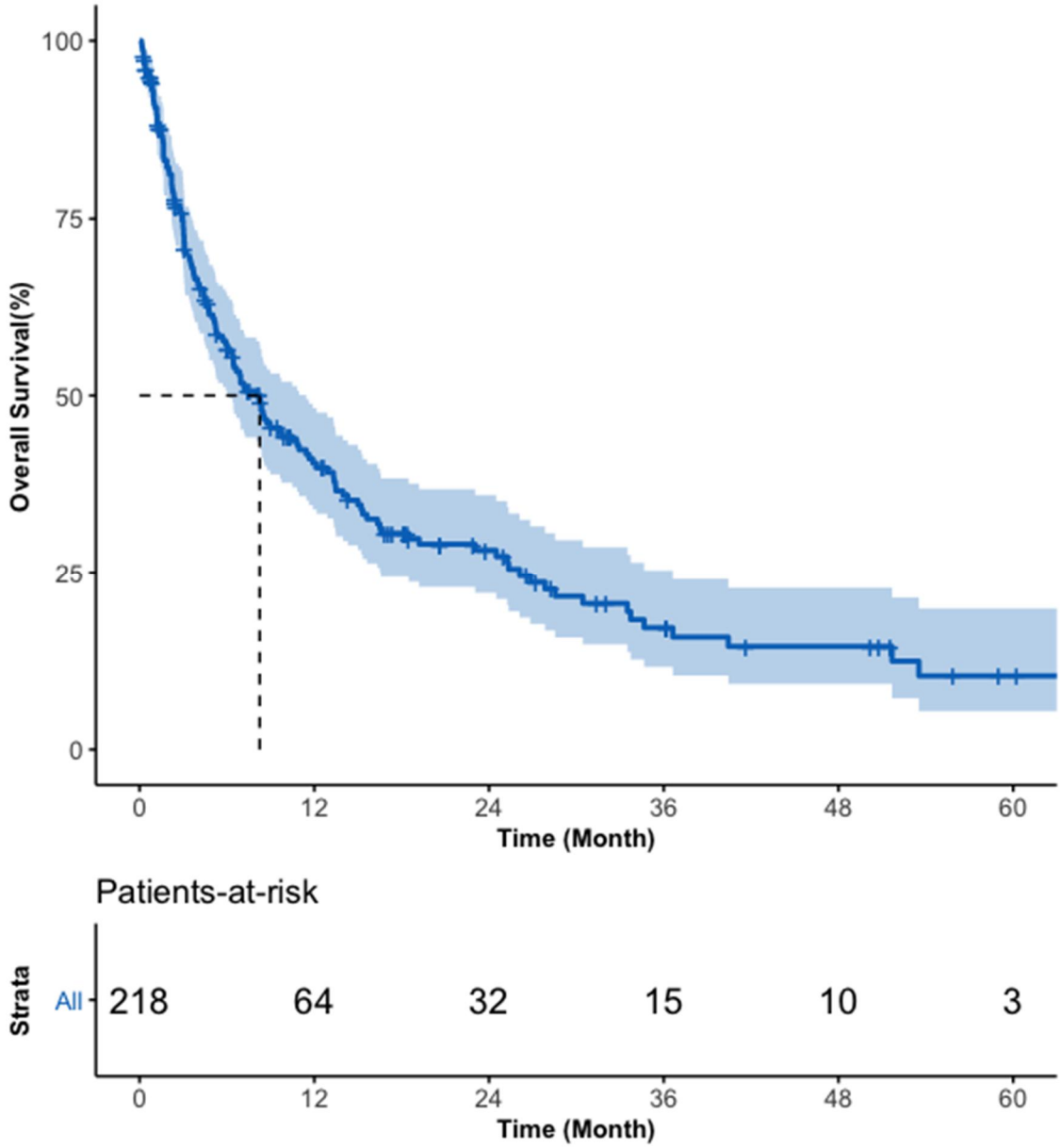
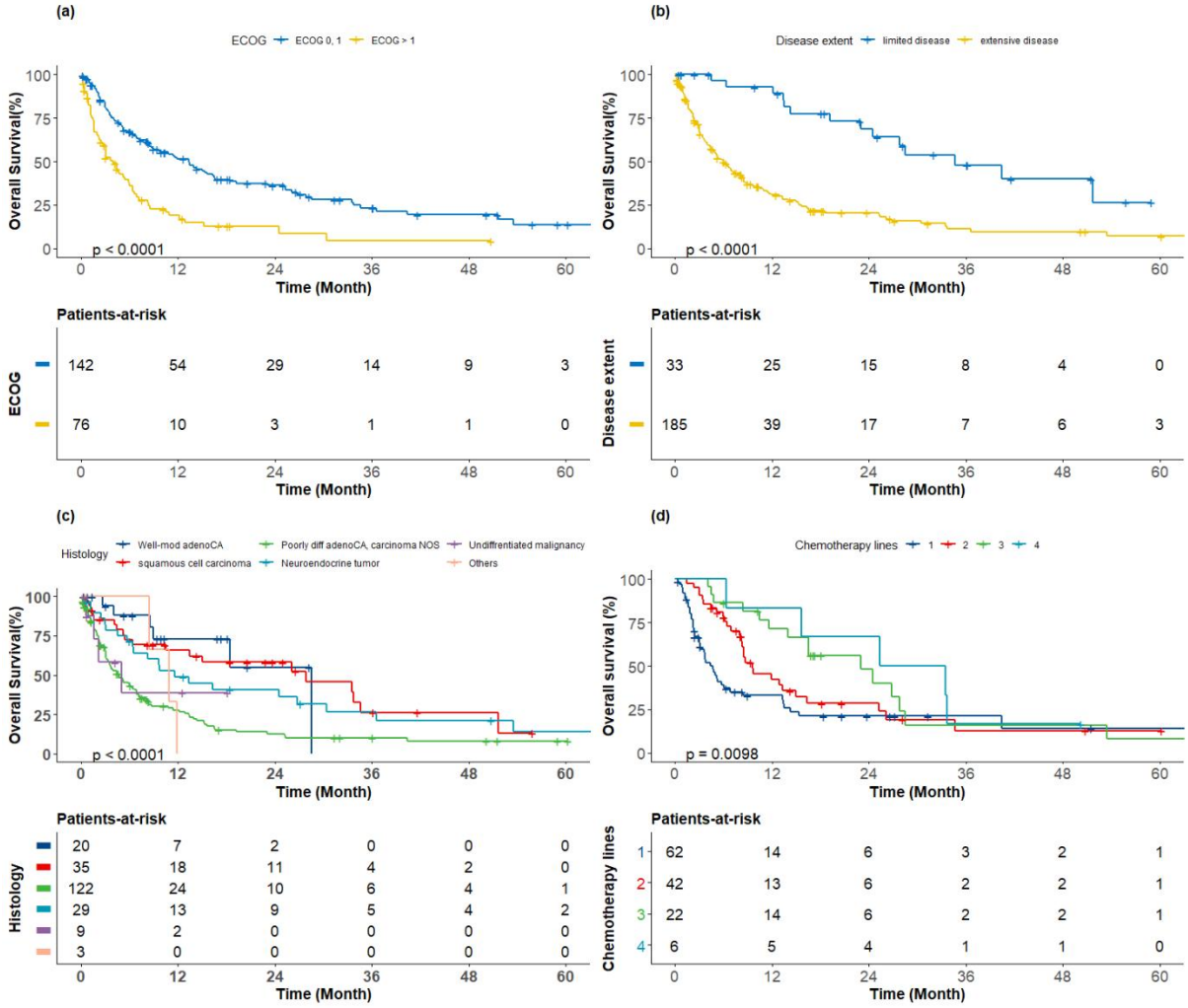


Figure 3 Overall survival by patient groups. (a) ECOG (b) Disease extent (c) Histologic groups (d) the number of chemotherapy lines



Discussion

In this study, most patients demonstrated initially metastatic disease, and commonly involved sites were lymph nodes, liver, bone, and lung. Ten percent of patients did not receive any anti-cancer treatment due to poor performance status and were treated with only best supportive care. Most patients received empirical cytotoxic chemotherapy, and surgery and radiation therapy play an auxiliary role in chemotherapy. Due to absence of a standard chemotherapy regimen for CUP, various types of regimens were administered. Among them, platinum-based chemotherapy was most common. Only a few patients were treated with immunotherapy and/or targeted therapy based on NGS results. The survival outcome was observed to be poor with standard treatment in a real-world setting.

Despite poor overall survival outcome of CUP, some subgroups of patients who had localized disease and treated with CCRT demonstrated favorable outcomes (median OS 51.7 months). Probably, it is because most of them were favorable subsets of CUP, such as squamous cell carcinoma involving cervical lymph nodes and inguinal adenopathy[14] and they showed good response to local treatment. Moreover, this may explain the reason why patients with squamous cell carcinoma showed better survival outcomes than patients with other histologic types.

Historically, some retrospective studies on treatment pattern and outcomes of CUP have been conducted. Löffler et al. analyzed 223 patients with CUP of adenocarcinoma or un-differentiated carcinomas in Germany and reported clinical characteristics, treatment patterns, and survival outcomes of CUP patients[15]. They reported that the most commonly involved organ system was the lymph node, liver, bone and lung. They analyzed ECOG PS, and the number of the metastatic organ systems was significantly related to survival outcomes, but age and sex did not show any such relation. These results were consistent with our findings, suggesting that PS and disease extent were important factors to predicting prognosis. It was different from our research in that it included only patients who were diagnosed with adenocarcinoma and poorly differentiated carcinoma. Interestingly, they showed better survival outcomes with a median OS of 16.5 months, which was better than the data of previous publications[14 16] and ours'. Considering that localized disease status was significantly associated with better survival outcome, it is possibly due to the differences in the proportion of patients with only single organ involvement (49% vs 15%).

Another large-scale study that included 4,562 patients using American Surveillance, Epidemiology, and End Results (SEER)–Medicare (SEER-M) linked database was conducted recently[17]. They showed recent trends in the diagnostic workup and treatment strategy in real-world settings with the analysis of patients' characteristics, use of diagnostic workup, and survival outcome.

Notably, a considerable proportion, i.e., 99 patients, received targeted therapy. The OS of all patients was poor, as the median OS was 1.2 months, and only 20.3% of patients were confirmed to be alive after 6 months. The reason for poor survival outcomes may be due to the relatively old age of patients and the low proportion of properly treated patients. In contrast, our study showed better survival of 8.2 months, with a higher proportion of patients receiving anticancer treatment. Even with recent advances in diagnostic methods and treatment strategies, the prognosis of CUP is still poor, as shown in our study. One of the limitations of that study was the exclusion of relatively young patients aged under 66 years.

To improve the diagnostic ability of CUP, a new approach was explored. GEP was developed to determine the primary site of CUP, and the results revealed excellent diagnostic benefits in tumor classification with approximately 85% accuracy, and it was comparable to immunohistochemistry[18 19]. However the clinical benefits of GEP have not yet been demonstrated[9 10], and currently it is not routinely recommended for diagnostic evaluation in CUP patients[20].

NGS, which was used to identify actionable mutation in patients with CUP, is widely used nowadays. Many studies based on NGS have been published recently, which presented a proportion of actionable gene mutations ranging from 30% to 85% in CUP patients[3 4 21-24]. This difference in the proportion of actionable gene mutation seems to be due to the difference of NGS assays, gene panels, and definition of actionable mutation in each study. In our study, we used the OncoKB data for classification[13], and 12 out of 23 people showed level 2 or 3 alternation, which was accounted for approximately 50% of the total.

With advances in the diagnostic procedure such as NGS, new treatment strategies are also suggested. Some studies conducted NGS in CUP patients, and they reported the possibility of personalized therapy based on NGS results[3 25]. In 2017, Varghese et al. reported the outcome of targeted therapy in patients with CUP, based on NGS results. Of the 150 patients who underwent NGS, 45 showed clinical genomic alteration, and 10% (n = 15) received targeted therapy, and the treatment outcome was very variable, as the time-to-treatment failure range was 1 month to 14 months[4]. In a study conducted in South Korea, among 21 patients who received NGS, 17 showed possible clinical genomic alteration and only one received targeted therapy[26]. More recently, phase 2 trial of site-specific therapy based on NGS results was conducted among 97 patients with CUP in October 2020, and it demonstrated that the 1-year survival probability was 53%, and the median OS was 13.7 months, which imply the possibility of the clinical application of tailored therapy[27]. Currently, the CUPISCO study (NCT03498521) is ongoing, which was a randomized trial comparing individualized targeted treatment or immunotherapy with standard platinum-based chemotherapy in

patients with CUP. The results of the study are expected to be released within a few years, drawing keen attention.

In South Korea, NGS was approved and reimbursed by the National Health Insurance service of Korea since March 2017 for patients with malignancies. In our study, 23 patients underwent NGS, and most of them were diagnosed since 2017. Of note, in our cohort, targeted therapy based on NGS results was provided to 2 patients and showed variable survival outcomes. One patient died despite 2 weeks of ipatasertib therapy, but the survival outcome of a patient who was treated with entrectinib was better than median OS of patients treated with standard empirical chemotherapy. The reason for our variable clinical outcomes may be explained by the small number of patients who received targeted therapy, and further large-scale prospective studies will be warranted to determine the role of targeted therapy.

This study has several limitations. First, this was conducted in a single center, and there may be selection bias due to nature of the single-center study, and our results may not be generalized for all CUP patients. The number of patients who were included in the study was small, but it is a relatively large-scale study considering the rare prevalence of the CUP, and there has not been a large-scale study of CUP in Asia, previously. Although, this was a retrospective study, it is meaningful because a prospective study cannot be easily conducted due to the nature of the disease and our results reflect the current trends and outcome, the real-world data. Another limitation is that we could not evaluate the adverse effects in each patient due to the heterogeneity of the chemotherapy regimen.

Conclusion

The prognosis of CUP was shown to be poor with standard treatment in a real-world setting. Patients with localized disease who received local treatment or those with a small number of metastasis and better PS who were treated with multiple lines of chemotherapy showed better survival. Novel targeted therapy based on NGS results will be expected to improve survival outcomes in the future, which warrants further investigation.

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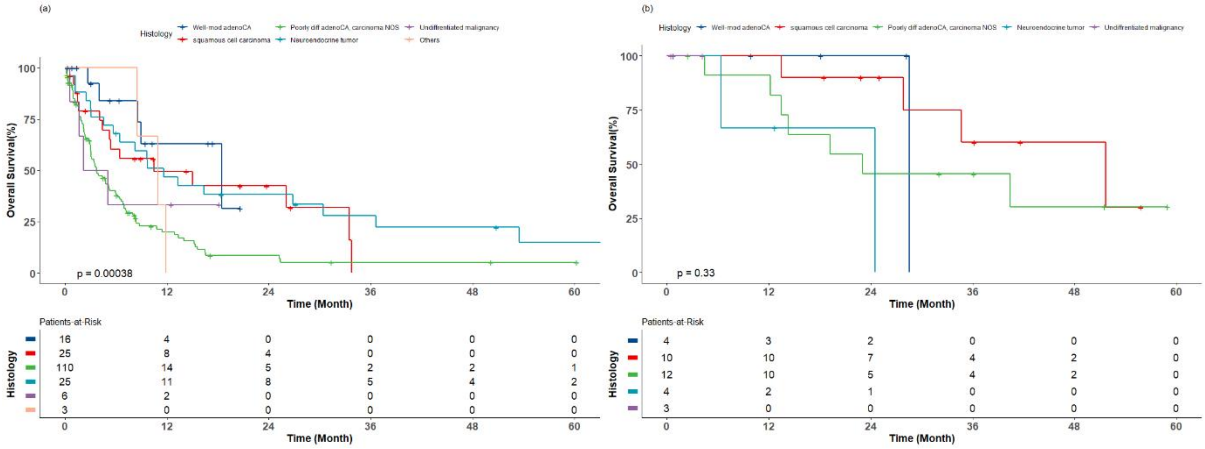
Table S1 Chemotherapy regimen of 3rd and 4th line (Supplement)

Chemotherapy regimen		
3rd line chemotherapy regimen	**n = 28**	
Clinical study	5	17.9
FP (5-FU, Cisplatin)	4	14.3
CAV(Cyclophosphamide, Doxorubicin and Vincristine)	1	3.6
VIP(Etoposide, Ifosfamide and Cisplatin)	1	3.6
GP**(Gemcitabine,** Carboplatin**)**	1	3.6
CAP(Cyclophosphamide, Adriamycin and Cisplatin)	1	3.6
CYVADIC(Cyclophosphamide, Vincristine, Doxorubin and Dacarbazine)	1	3.6
Docetaxel	1	3.6
IP(Irinotecan, Cisplatin)	3	10.7
Others*	10	35.7
4th line chemotherapy regimen	**n = 6**	
Gemcitabine	1	16.6
Ipatasertib	1	16.6
VIP(Etoposide, Ifosfamide and Cisplatin)	1	16.6
CAV(Cyclophosphamide, Adriamycin and Cisplatin)	1	16.6
PC(Paclitaxel, Cisplatin)	1	16.6
Pembrolizumab	1	16.6

*includes EMF(1), MVAC(1), NP(1), ifosfamide(1), FOLFIRI(1), AP(1), IT-MTX(1), bevacizumab/doxorubicin(1), AIM(1) and MF(1)

Figure S1 Overall Survival (%) by histology (a) patients with extensive disease and (b) patients with localized disease (Supplement)



국문요약

연구배경 : 원발부위 미상암(Cancer of unknown primary, CUP) 이란 표준적인 검사 과정에도 불구하고 암종의 원발부위를 찾지 못한 전이성 종양을 의미한다. 예후는 대체적으로 나쁘며, 일반적인 중앙 생존기간은 약 6개월에서 12개월이다. 원발부위 미상암에 대한 표준적인 치료 방법은 아직 확립되지 않았으며 경험적인 세포독성항암화학요법으로 치료하는 경우가 많다. 최근의 진단적, 치료적 방법의 발전에도 불구하고, 원발부위 부위 미상암에서의 임상적 효용은 명확하지 않다. 실제 임상에서의 원발부위 미상암의 진단 및 치료 방법에 대해 알아보기 위하여 본 연구에서는 원발부위 미상암 으로 진단 받은 환자들을 대상으로 후향적 코호트 연구를 시행하였다.

연구방법 : 2009년 1월부터 2019년 12월 까지 총 218 명의 환자가 서울아산병원에서 원발부위 미상암으로 진단 받았으며 이 들을 대상으로 연령, 성별, 신체 활동 능력 (Eastern cooperative oncology group performance status, ECOG PS), 조직검사 결과, 전이 부위의 수 및 위치, 치료 방법, 항암을 시행하였을 경우 항암 시행 횟수 및 요법, 항암제에 대한 반응 정도, 생존 기간 등에 대한 분석을 시행하였다. 또한 차세대 염기서열 분석법(Next generation sequencing, NGS)을 시행한 경우 이에 대한 분석도 포함하였다.

연구결과 : 진단 당시 연령의 중앙값은 62세 (범위 19-91) 이었으며 62.3%의 환자가 남성 이었다. 85%의 환자는 진단 초기부터 다발성 장기 침범을 보였다. NGS를 시행한 환자는 22명 (10.09%) 이었으며 이 들 중 3명에서 임상적으로 의미있는 변이(actionable clinical alteration) 를 보였다. 60.3% 환자가 경험적 세포독성항암화학요법으로 치료 받았으며, 각각 2명의 환자가 면역요법(immunotherapy) 및 표적치료(targeted therapy)를 받았다. 46명(21.1%) 의 환자가 수술을 받았으며, 66명(30.2%)의 환자가 방사선 치료를 받았다. 모든 환자의 중앙 생존기간은 8.25 개월 이었으며, (95% 신뢰구간 [Confidence interval, CI] 6.18-11.44) 항암화학요법을 시행받은 환자들의 무진행생존기간[Progression-free survival, PFS] 은 4.37 개월이었다(95% CI 3.35-5.33). 다변량 콕스 회귀 분석에서(Multivariate cox regression analysis) 2개 이하의 전이 부위(위험비 [Hazard ratio, HR] 2.69), 신체활동능력 (ECOG PS) 0 혹은 1 (HR

2.47), 국소질환(HR 3.71) 의 경우 생존율에 유의한 영향을 미치는 요소로 나타났다.

연구결론 : 실제 임상에서 경험적 세포독성항암화학요법으로 치료한 원발부위 미상암의 예후는 여전히 불량한 것으로 나타났다. 일부 국소부위 혹은 2 곳 이하로 전이된 암종, 신체활동능력이 비교적 좋으며 항암치료를 수 차례 시행 받은 환자들의 경우 예후가 좋은 편이었다. NGS 결과를 바탕으로 한 표적치료제 등은 추후 원발부위 미상암의 예후를 개선시켜줄 것으로 생각되며 추후 이에 대한 추가적인 연구가 필요할 것으로 생각된다.