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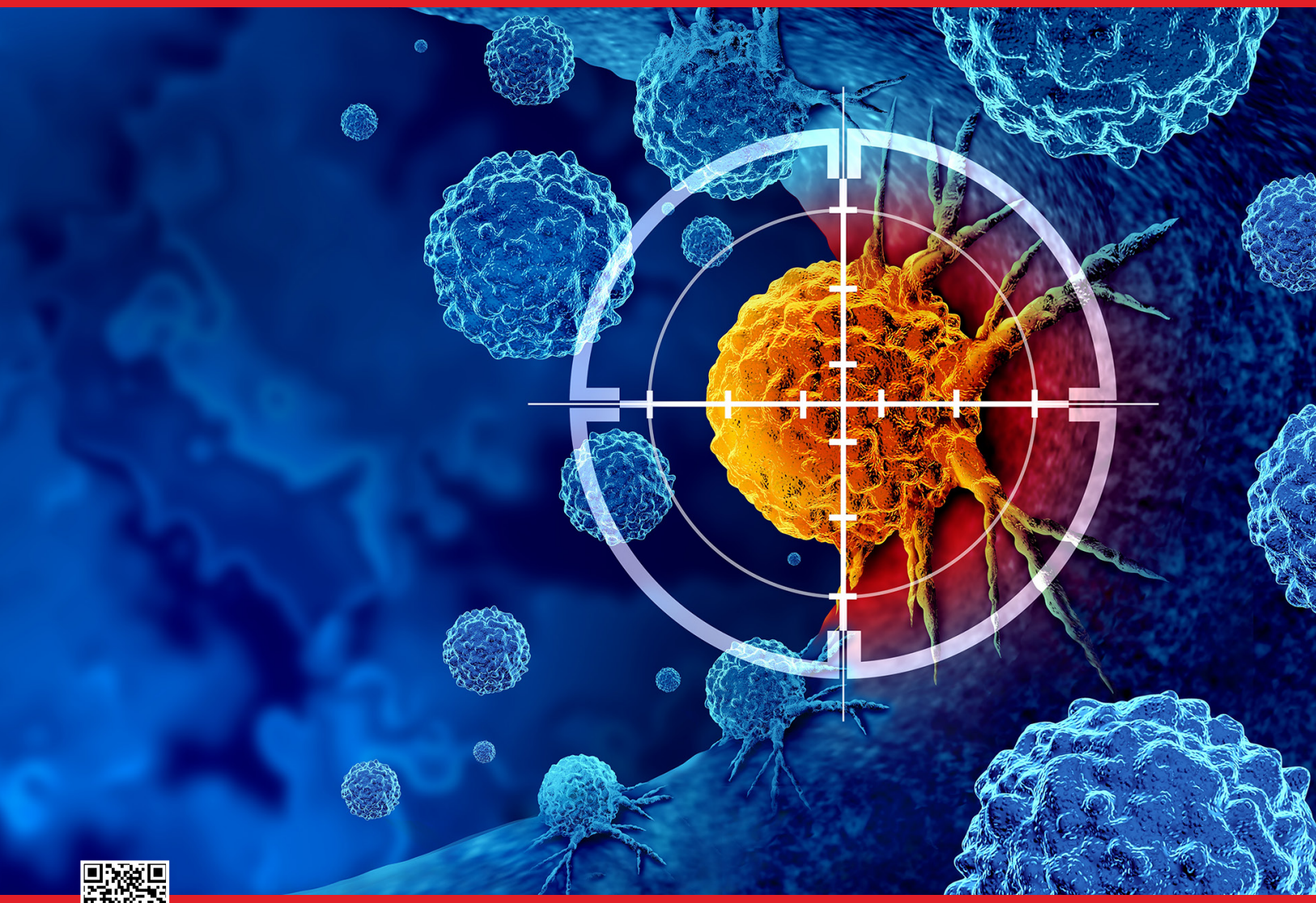

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ORIGINAL ARTICLE

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J Oncol Sci 2025;11(1):1-6

CDK4-6 Inhibitors: Evaluation of Efficacy in Cases of Hormone Receptor-Positive HER2-Negative Breast Cancer with Only Bone Metastasis

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ABSTRACT

Objective: Bone is the most common site of metastasis in patients with hormone receptor (HR)-positive breast cancer. However, 17-37% of these patients with metastatic disease develop metastasis only in the bone. In this context, the present study aimed to compare the CDK4-6 inhibitors palbociclib and ribociclib in terms of their efficacy in treating HR-positive human epidermal growth factor receptor 2 (HER-2)-negative breast cancer patients with only bone metastases detected at diagnosis.

Material and Methods: The study was conducted as a retrospective observational study of 31 patients with HR-positive and HER2-negative breast cancer and only bone metastases who were treated with CDK4-6 inhibitors. The patients were divided into two groups based on the CDK4-6 inhibitor used and subjected to overall survival (OS) analysis.

Results: The median age of the patients included in the present study was 57 years (36-76). The median OS in the ribociclib group was 25.46 months [confidence interval (CI) was not reached in the Kaplan-Meier analysis]. The median OS in the palbociclib group was 16.07 months (95% CI: 7.88-24.25). The difference in OS between the two groups was statistically significant (p=0.043). Among the other variables with the potential of affecting the OS of these patients, the N stage and survival values were observed to be significantly different (p=0.033) between the two groups. The multivariate analysis revealed the N stage (p=0.011) and the type of CDK4-6 inhibitor used (p=0.023) as the independent risk factors that affected the OS of these patients.

Conclusion: In patients with hormone-positive HER2-negative breast cancer with only bone metastasis, ribociclib administration achieved increased OS compared to the use of palbociclib.

Keywords: Breast cancer; hormone-positive cancer; bone metastasis; CDK4-6 inhibitors

INTRODUCTION

Breast cancer is the most frequently detected cancer in women worldwide and also the most common cause of death caused by cancer in women.[1] Approximately 80% of the patients with breast cancer are hormone receptor (HR)-positive at the time of diagnosis.[2] The introduction of endocrine therapies has particularly increased survival in metastatic HR-sensitive breast cancer. Endocrine therapies are less toxic compared to chemotherapy while leading to similar survival rates, due to which these therapies are used as the first-line treatment of these patients.[3]

The most effective and recommended first-line endocrine therapy is the use of a combination of a cyclin-dependent kinase (CDK) 4-6 inhibitor, such as palbociclib, ribociblib, and abemaciclib, and an aromatase inhibitor (AI) or tamoxifen (TMX) along with luteinizing hormone-releasing hormone (LHRH) analogs.[4-7] Few studies have, however, demonstrated

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that the efficacy of one of these CDK4-6 inhibitors is superior to the others. However, the drug side effect profiles of these agents are slightly different, and patient comorbidities should be considered when using these drugs for treatment.

Bone is the most common organ to which HR-positive breast cancer cells have been observed to be metastasized.[8] According to the autopsy results of patients diagnosed with breast cancer, approximately 70% of these patients develop bone metastasis.[9] In contrast, cases of only bone metastasis are scarce, accounting for just 17%-37% of patients with metastatic disease.[10] Moreover, this group of patients is reported to have a much better prognosis than the patients with bone metastases along with other systemic metastases.[11]

The present study aimed to compare the CDK4-6 inhibitors palbociclib and ribociclib in terms of their effectiveness in treating patients with HR-positive breast cancer with only bone metastasis detected at the time of diagnosis.

MATERIAL AND METHODS

The present study was designed as a retrospective observational study that enrolled 31 patients who were admitted to our clinic between May 2019 and June 2023, were older than 18 years, had only bone metastasis at the time of diagnosis, were HR-positive in biopsy results, were HER2-negative, and administered CDK4-6 inhibitors as treatment. The patients with no metastasis detected at the time of diagnosis, a non-bone metastasis, age less than 18 years, HER2-positivity, and not treated with CDK4-6 inhibitors were excluded from the study. Since the earliest response imaging examinations of the patients were conducted in the third month after the commencement of treatment, each patient received CDK4-6 inhibitors for at least three months. Since all patients in the study had bone metastasis, all of them received either zolendronic acid or denosumab. All retrospective data on clinical characteristics, pathology and laboratory results, and treatment data were retrieved from the medical records of patients. The limit values used in our laboratory were used as threshold values for the laboratory parameters. A receiver operating curve (ROC) analysis was conducted to determine the threshold values of estrogen receptor (ER), progesterone receptor (PR), and Ki-67. The time between the commencement of treatment and death due to any cause was utilized to determine the overall survival (OS) of the patients.

Since the study was designed as a retrospective one, the study was approved by the Ankara Bilkent City Hospital Ethics Committee for Clinical Research at our Hospital (date: February 28, 2024, no: 24-33) without the requirement of obtaining informed consent from the patients. The study was conducted in accordance with the Declaration of Helsinki.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 25 (USA). Normal distributions were determined using histograms and the Shapiro-Wilk test. Continuous variables with normal distribution were expressed as means ± standard deviations, while the variables with a non-normal distribution were expressed as median (minimum-maximum) values. The continuous variables were compared between the two groups using the Mann-Whitney U test. A chi-squared or Fisher's exact test was conducted to compare categorical variables. The threshold values were determined based on the ROC analysis. Kaplan-Meier and Cox regression analyses were performed for survival and prognostic factors. $P<0.05$ was considered the threshold of statistical significance.

RESULTS

The median age of patients in the present study was 57 years (age range 36 to 76 years). The median follow-up period was 13.67 months (4.11 to 39.13 months). A total of 11 (35.5%) patients among all the patients who participated in the study died during the follow-up period. The median duration of the usage of CDK4-6 inhibitors was 12.9 (4.1 to 39.13) months.

The ROC analysis revealed the following threshold values for ER, PR, and Ki-67: 91% for the ER percentage [area under curve (AUC): 0.564, sensitivity: 50.0%, specificity: 54.5%, p=0.563], 67.5% for the PR percentage (AUC: 0.634, sensitivity: 60.0%, specificity: 63.6%, p: 0.223), and 22.5% for Ki-67 (AUC: 0.655, sensitivity: 65.0%, specificity: 63.6%, p=0.16). The insignificant p-values in the ROC analysis could be explained by the small sample size of the study.

Further, 28 patients (90.3%) among all patients included in the study received CDK4-6 inhibitors as the first-line treatment, while 3 patients (9.7%) received this treatment as the second-line treatment. A total of 14 (45.2%) patients received ribociclib, while 17 patients (54.8%) received palbociclib. None of the patients had undergone surgery for their primary breast tumor. Three patients (9.7%) were subjected to palliative radiotherapy for the bones. The baseline characteristics of patients are presented in Table 1.

The patients who received ribociclib or palbociclib were divided into two groups and compared in terms of their age, clinical T stage, clinical N stage, ER percentage, PR percentage, pathologic grade, Ki-67 percentage, CEA, and CA15.3. The comparative analysis revealed no statistically significant differences between the two groups in any of the variables (Table 2). Further, for supportive bone therapy, 23

TABLE 1: Baseline characteristics of all patients.	
Age, years	57 (36-76)
Eastern Cooperative Oncology Group, performance status	
0	14 (45.2%)
1	15 (48.4%)
2	2 (6.5%)
T stage	
1-2	18 (58.1%)
3-4	13 (41.9%)
N stage	
0-1	8 (25.8%)
1-2	23 (74.2%)
Estrogen receptor percent	
≥90	22 (71.0%)
<90	9 (29.0%)
Progesterone receptor percent	
≥67	16 (51.6%)
<67	15 (48.4%)
Kİ-67	
≥22.5	17 (54.8%)
<22.5	14 (45.2%)
Carcinoembryonic antigen	
≥2.5	17 (56.7%)
<2.5	13 (43.3%)
CA15.3	
≥32.4	16 (53.3%)
<32.4	14 (46.7%)
Radiotherapy to bone	
Yes	3 (9.7%)
No	28 (90.3%)
Time to treatment with CDK4-6 inh	
First line	28 (90.3%)
Second line	3 (9.7%)
CDK4-6 inhibitor	
Ribociclib	14 (45.2%)
Palbociclib	17 (54.8%)
CA: Cancer antigen; CDK: Cyclin-dependent kinase.	

(74.2%) patients received zolendronic acid, and 8 (25.8%) patients received denosumab. Denosumab treatment was administered to 4 patients in the ribociclib group (28.6%) and 4 patients in the palbosiclib (23.5%) group.

The median OS in the ribociclib group was 25.46 months (confidence interval was not reached in the Kaplan-Meier analysis). The median OS in the palbociclib group was 16.07 months (95% CI: 7.88 to 24.25). There was a statistically significant difference between the two groups (p=0.043) (Figure 1).

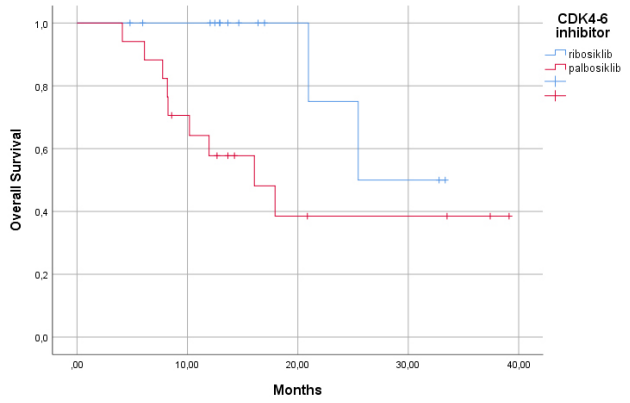

FIGURE 1: Kaplan-Meier survival curves for overall survival of CDK4-6 inhibitor groups.

CDK: Cyclin-dependent kinase

The other variables that could affect OS, such as age (p=0.791), clinical T stage (p=0.059), ER percentage (p=0.323), PR percentage (p=0.301), tumor grade (p=0.945), Ki-67 in pathology (p=0.194), CEA level (p=0.417), CA15.3 level (p=0.251), and the line of treatment in which the CDK4-6 inhibitor was used (p=0.932), were not significantly different between the groups. Only the clinical N stage variable presented a statistically significant difference with OS (p=0.033). The multivariate analysis revealed the N stage (p=0.011) and the type of CDK4-6 inhibitor used (p=0.023) as the independent risk factors affecting OS (Table 3).

DISCUSSION

The present study aimed to evaluate the effectiveness of CDK4-6 inhibitors in patients with HR-positive and HER2-negative breast cancer with only bone metastasis. According to the results of the study, the use of ribociclib increased OS compared to the use of palbociclib in these patients.

Recent advances in endocrine therapies have led to the adoption of the combination of CDK4-6 inhibitors and TMX or AI along with LHRH analogs as the standard of care in the initial treatment of patients with metastatic HR-positive and HER2-negative breast cancer, except for patients with visceral crisis. This treatment modality leads to an efficacy similar to that achieved using chemotherapy while the side effects are considerably reduced.[12] In the present study, all patients were treated with the CDK 4-6 inhibitor ribociclib or palbociclib, and most of these patients received the drugs as first-line treatment.

Several previous studies have compared the efficacy of different CDK4-6 inhibitors in patients with metastatic HR-positive breast cancer. Zhao et al.[13] indirectly compared the patients participating in the PALOMA-2, MONALEESA-2,

TABLE 2: Association between the CDK4-6 inhibitors and features of patients.

Variables	CDK4-6 inhibitor		p value
	Ribociclib: n, (%)	Palbociclib: n, (%)	
Age			
≤58	7 (50)	9 (52.9)	0.999
>58	7 (50)	8 (47.1)	
T stage			
1-2	11 (78.6)	7 (41.2)	0.067
3-4	3 (21.4)	10 (58.8)	
N stage			
0-1	4 (28.6)	4 (23.5)	0.999
2-3	10 (71.4)	13 (76.5)	
Estrogen receptor percent			
≥90	10 (71.4)	9 (52.9)	0.461
<90	4 (28.6)	8 (47.1)	
Progesterone receptor percent			
>67	8 (57.1)	8 (47.1)	0.722
≤67	6 (42.9)	9 (52.9)	
Grade			
1-2	13 (92.9)	11 (64.7)	0.062
3	1 (7.2)	6 (35.3)	
Ki-67			
<22.5	5 (35.7)	9 (52.9)	0.473
≥22.5	9 (64.3)	8 (47.1)	
Carcinoembryonic antigen			
<2.5	7 (53.8)	6 (35.3)	0.460
≥2.5	6 (46.2)	11 (64.7)	
CA15.3			
<32.4	7 (53.8)	7 (41.2)	0.713
≥32.4	6 (46.2)	10 (58.8)	

CA: Cancer antigen; CDK: Cyclin-dependent kinase.

and MONARCH-3 trials and reported no difference in OS or PFS between patients receiving ribociclib, palbociclib, or Abemaciclib. Xie et al.[14] reported no difference in OS or PFS between the different CDK4-6 inhibitor subtypes in 4,580 patients. No study in the literature has, to the best of the author's knowledge, demonstrated to date that either of the above two drugs leads to better outcomes in terms of OS than the other. However, in the recently reported results of the survival analyses from PALOMA and MONALEESA trials, no statistically significant difference in OS was stated upon the use of palbociclib, while OS was significantly higher with the use of ribociclib.[15,16] In the present study, as well, a higher OS was observed in patients who received ribociclib.

A meta-analysis of patients with HR-positive and HER2-negative breast cancer with only bone metastasis revealed that the treatment of choice should be the same as the one used for patients with other metastatic hormone-positive cancers.[17] Survival in these patients is better than that in patients with bone metastasis who also have visceral metastases.[18] Studies have demonstrated that variables such as previous use of bisphosphonate, presence or absence of symptoms, number of bone metastases, and treatment modalities affect survival in this group of patients.[11,18] No study has, to the best of the author's knowledge, compared the efficacy of CDK4/6 inhibitors in these patients to date. The present study demonstrated that in this group of patients, the use of ribociclib leads to better OS than the use of palbociclib.

TABLE 3: Prognostic factors of overall survival in patients.	
Univariable analysis	
Variables	**p value**
Age, ≤50 vs. >50	0.791
Clinical T stage, 1-2 vs. 3-4	0.059
Clinical N stage, 0-1 vs. 2-3	**0.033**
Estrogen receptor percent, <90 vs. ≥90	0.323
Progesterone receptor percent, <67 vs. ≥67	0.301
Grade, 1-2 vs. 3	0.945
Ki-67, <22.5 vs. ≥22.5	0.194
Carcinoembryonic antigen, <2.5 vs. ≥2.5	0.417
CA15.3, <32.4 vs. ≥32.4	0.251
Line of CDK4-6 inh, first vs second	0.932
CDK4-6 inh, ribociclib vs palbociclib	**0.043**
Multivariable analysis	
Variables	**p value**
N stage, 0-1 vs 2-3	**0.011**
CDK4-6 inh, ribociclib vs palbociclib	**0.023**
CA: Cancer antigen; CDK: Cyclin-dependent kinase; Inh: Inhibitor.	

Study Limitations

Certain limitations of the present study include the single-center setting, the small sample size, and the retrospective design.

CONCLUSION

Survival in patients with hormone-positive and HER2-negative breast cancer with only bone metastasis is better than that in other breast cancer groups. However, the literature on which drugs to select for this group of patients is scarce. In the present study, the use of ribociclib for this patient group resulted in much better OS than the use of palbociclib. However, larger studies have to be conducted to assess the effectiveness of different treatments in patients with hormone-positive and HER2-negative breast cancer with only bone metastasis.

Ethics

Ethics Committee Approval: Since the study was designed as a retrospective one, the study was approved by the Ankara Bilkent City Hospital Ethics Committee for Clinical Research at our Hospital (date: February 28, 2024, no: 24-33).

Informed Consent: The participants provided written informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: B.C., D.U., Concept: S.S., Design: S.S., G.U., Data Collection or Processing: S.S., D.B., Analysis or Interpretation: M.M., B.C., Literature Search: Ö.B., E.A., Writing: S.S., M.M., Critical Review: F.T.K.

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ORIGINAL ARTICLE

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Breast Radiotherapy: A Potential Risk Factor for Resistant Clone Development in Patients with Brain Metastasis

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ABSTRACT

Objective: The human brain is a frequent site of breast cancer metastasis. The various therapeutic approaches for treating brain metastases include surgical intervention, stereotactic radiosurgery (SRS), and whole-brain radiotherapy (WBRT). However, the literature on the association between prior breast RT and the effectiveness of intracranial RT subsequent to treatment is scarce. The present study, therefore, aimed to understand the association between previous breast RT and intracranial progression-free survival (iPFS).

Material and Methods: In the present study, the relationship of epidemiological, pathological, and clinical features, especially previous breast RT, with iPFS was explored in the patients diagnosed with human epidermal growth factor receptor 2-positive breast cancer along with brain metastasis. These patients did not undergo surgery for brain metastasis and received WBRT/SRS instead.

Results: Fifty-one patients were included in the present study. The median age of these patients was 46 years. Among the included patients, 20 patients had previously undergone whole breast or chest wall RT. In 19 patients, SRS was utilized rather than WBRT. The iPFS was significantly shorter in patients who had previously received RT for the primary lesion compared to those who had not received RT (mPFS: 7.96 vs. 14.56 months, p=0.002, hazard ratio: 3.06, confidence interval: 1.52-6.12). No relationships of iPFS with the treatments used prior to RT, type of RT, sites of metastasis during RT, systemic therapy administered after RT, and status of *de novo* metastatic/recurrent disease were noted.

Conclusion: Patients who had undergone previous RT to the locoregional region exhibited significantly poorer iPFS following the RT performed for brain metastasis.

Keywords: Breast radiotherapy; HER-2 positive breast cancer; brain metastasis

INTRODUCTION

The human brain is a frequent site of metastasis in solid organ malignancies, and approximately 25% of the patients with cancer eventually develop brain metastases.[1] The most common tumor types that tend to metastasize to the brain include malignant melanoma, lung cancer, and breast cancer.[2] After the development of brain metastasis, the overall survival (OS) duration is generally less than 12 months.[3] Immun checkpoint inhibitors and certain tyrosine kinase inhibitors have demonstrated high efficacy in treating brain metastases.[4-8] These treatments have led to improved survival rates, particularly among patients with lung cancer and malignant melanoma, along with brain metastases.

Breast cancer is the most commonly diagnosed cancer among women worldwide and the second leading cause of cancer-related deaths after lung cancer.[9] Despite the advances in

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systemic therapy for breast cancer, which have significantly improved the survival rates of patients, a corresponding increase has been noted in the incidence of brain metastases.[10-13] Brain metastases have been observed more frequently in patients with hormone receptor (HR)-negative and human epidermal growth factor receptor 2 (HER2)-positive breast cancer.[14] While certain studies have indicated that trastuzumab treatment delayed the development of brain metastases, a previously reported meta-analysis revealed an increased probability of brain metastasis at the time of the first relapse.[15,16]

The standard treatment options for patients with breast cancer who develop brain metastasis include surgery, stereotactic radiosurgery (SRS), and whole-brain radiotherapy (WBRT).[17]

Surgical interventions for metastasis are prioritized less and are recommended mainly in cases of advanced disease where systemic control cannot be achieved or in patients who are unable to undergo surgery. In such patient populations, whole-brain RT or SRS are often used as the primary treatment options, depending on the number of metastatic lesions detected in the brain. However, not all patients respond to RT, and previous studies have explored the factors responsible for this primary resistance to RT in certain patients.[18]

In the above context, the author of the present report hypothesized that prior RT to the primary cancer site could enable the suppression of radio-sensitive clones while allowing the survival of radio-resistant clones. No study reported in the existing literature has, to the best of the author's knowledge, specifically investigated the impact of prior RT to the primary cancer site on the outcomes of the subsequent RT treatment for brain metastasis. Therefore, the present study aimed to explore the factors, including prior breast RT, that impact the effectiveness of brain RT in patients diagnosed with HER2-positive breast cancer along with brain metastasis.

MATERIAL AND METHODS

The present study was designed as a retrospective study conducted with patients who visited the outpatient clinics of Hacettepe University Oncology Hospital between January 2018 and January 2024. The inclusion criteria for the study were as follows: a diagnosis of metastatic breast cancer with positive HER2 expression, presence of brain metastasis, absence of surgical intervention for brain metastasis, and receipt of RT for brain metastasis. The exclusion criteria were as follows: the presence of brain metastasis at the time of breast cancer diagnosis, medical oncology or radiation oncology follow-up at another medical center, and lack of response evaluation imaging after RT (except for the cases in which the patient died prior to performing imaging control, which were, therefore, included in the study). The patients with five or more brain metastases received WBRT as the initial treatment modality, with a fraction dose of 3 Gy to a total dose of 30 Gy. However, for patients with less than five metastases, especially those with controlled primary cancer and no other metastasis, SRS was preferred as the treatment approach.

The clinical data (age, stage, pre/post RT anti-HER2 therapy, number of brain metastases, type of RT, and the site of metastasis during RT) and the pathological characteristics (estrogen receptor expression) of all included patients were documented, and prognostic factors were investigated, including whether a relationship existed between the time to intracranial progression-free survival (iPFS) and previous breast RT. The definition of iPFS was as follows: the duration between the initiation of RT and the radiologically confirmed intracranial progression or death.

Statistical Analysis

Statistical analysis was conducted using the IBM SPSS Statistics Version 22 (Chicago, IL, USA) software package. The relationship between various clinical factors and brain PFS was assessed based on Kaplan-Meier curves. Median survival times along with their corresponding 95% confidence intervals (CI) were reported. Cox's regression analysis could not be performed due to the limited number of patients included in the study. A p-value of less than 0.05 was considered statistically significant.

Ethical approval for the study was obtained from the Local Research Ethics Committee of the Faculty of Medicine at Hacettepe University (date: January 24, 2023, no: GO/2308). All procedures and stages of the study were conducted in compliance with the ethical principles outlined in the World Medical Association Declaration of Helsinki, which governs the inclusion of human subjects in medical research. The participants provided written informed consent.

RESULTS

Baseline Characteristics

Fifty-one patients were enrolled in the present study. The median age was these patients was 4610.52± years, and 25 of these patients had estrogen receptor-positive tumors. At the time of diagnosis, 9 among the included 51 patients had Stage 2, 12 had Stage 3, and 30 had Stage 4 disease. Among all patients, 20 had undergone whole breast/CW with or without regional RT previously, while 31 had not received locoregional

RT. All patients had received treatment with trastuzumab, while 11 had received pertuzumab, 7 had received TDM-1, and 2 had received lapatinib.

Brain metastasis was detected with a single focus in 7 patients, 2-4 foci in 12 patients, and 5 or more foci in 32 patients. SRS was performed for 19 patients, while whole-brain RT was conducted for 32 patients. At the time of brain radiation therapy, liver metastasis was detected in 12 patients, lung metastasis in 14 patients, and bone metastasis in 26 patients. After RT, eight patients received the capecitabine-lapatinib combination, 12 received TDM1, and 31 received the trastuzumab+chemotherapy±pertuzumab treatment. The basal epidemiological, clinical, and pathological characteristics of all patients are presented in Table 1.

Clinical and Pathological Characteristics of the Patients Who Received and Those Who Did Not Receive Breast RT

The mean age at diagnosis was 47.35±11.60 years for patients who received breast RT and 45.00±9.68 years for those who did not receive breast RT. The duration between the diagnosis and the development of brain metastasis was 22.46±40.35 months for patients who received breast RT and 18.10±10.14 months for those who did not receive breast RT. Estrogen receptor positivity was similar in both groups. At the time of diagnosis, 6 patients (30%) who received breast RT were classified as Stage 2, 7 (35%) as Stage 3, and 7 as Stage 4, while in the group that did not receive breast RT, 3 patients were classified as Stage 2 (9.7%), 5 as Stage 3 (16.1%), and 23 as Stage 4 (74.2%) (p=0.020). The treatments received prior

TABLE 1: Baseline characteristics of patients.

			No (%)
Age ($\overline{X}$ ± SD)		46.00±10.52	
Estrogen receptor expression Negative		Positive	25 (49)
		26 (51)	
Stage at diagnosis		2	9 (17.6)
		3	12 (23.55)
		4	30 (58.8)
Breast RT No		Yes	20 (39.2)
		31 (60.8)	
Prior anti-HER2 therapy	Trastuzumab	Yes	51 (100)
		No	0 (0)
	Pertuzumab	Yes	11 (21.6)
		No	40 (78.4)
	Ado-trastuzumab emtansine	Yes	7 (13.7)
		No	44 (86.3)
	Lapatinib	Yes	2 (3.9)
		No	49 (96.1)
Brain metastasis number		1	7 (13.7)
		2-5	12 (23.5)
		>5	32 (62.7)
Treatment after RT	Capecitabine+Lapatinib		8 (17.6)
	Ado-trastuzumab emtansine		12 (23.5)
	Trastuzumab+Cht+Pertuzumab		31 (58.8)
RT type	Stereotactic radiosurgery		19 (37.3)
	Whole brain RT		32 (62.7)
During brain RT	Liver metastasis	Yes	12 (23.5)
		No	39 (76.5)
	Lung metastasis	Yes	14 (27.5)
		No	37 (72.5)
	Bone metastasis	Yes	26 (49)
		No	25 (51)

SD: Standard deviation; RT: Radiotherapy; Cht: Chemotherapy; HER2: Human epidermal growth factor receptor 2.

to brain RT were similar in both groups. All patients received treatment with trastuzumab, while among those who received breast RT, 3 (15%) received pertuzumab, 3 (15%) received TDM-1, and 1 (5%) received lapatinib. In patients who did not receive breast RT, the usage rates of pertuzumab, TDM-1, and lapatinib prior to brain metastasis were 25.8%, 12.9%, and 3.2%, respectively, which were similar to those noted for the patients who received breast RT (p-values: 0.493, 1.000, and 1.000, respectively).

Brain-Progression Free Survival and OS

The median follow-up period in the study population was 25.10±4.82 months, and during this period, progression of brain lesions was observed in 40 patients. The median brain PFS was 11.90±0.92 months in the study population. Brain PFS was significantly shorter in patients who had received RT to the primary lesion previously, compared to the patients who had not received this treatment (mPFS: 7.96 months vs. 14.56 months, p=0.002, HR: 3.06, CI: 1.52-6.12; the relationship between the iPFS of patients who received and did not receive adjuvant RT is depicted in Figure 1). No significant relationship was noted between the PFS of brain lesions and the treatments used prior to RT [mPFS: 11.6 vs. 11.90 months, p=0.633, hazard ratio (HR): 0.80, CI: 0.33-1.95 for pertuzumab; mPFS: 11.90 vs. 12.16 months, p=0.428, HR: 0.69, CI: 0.28-1.70 for TDM-1; mPFS: 21.10 vs. 11.90 months, p=0.25, the number of brain metastases (<5 vs. ≥5); mPFS: 11.9 vs. 12.16 months, p=0.428, HR: 0.69, CI: 0.28-1.70], the type of RT (whole brain RT vs. SRS) (p=0.575, HR: 0.83, CI: 0.43-1.58), other sites of metastasis during RT (p=0.411 HR: 0.72 CI: 0.33-1.56; p=0.772, HR: 1.10 CI: 0.54-2.24; p=0.446, HR: 1.27, CI: 0.67-2.40 for liver, lung, and bone, respectively), systemic therapy administered after RT (mPFS: 19.30 months, 95% CI: 14.77-23.82, mPFS: 11.76 months 95%, CI: 7.52-16.00, mPFS: 10.46 months, 95% CI: 6.84-14.09, p=0.081, for TDM1, trastuzumab+chemotherapy±pertuzumab, and capecitabine-lapatinib treatments, respectively). In the subgroup analysis of the 30 patients diagnosed with *de novo* metastatic breast cancer, the brain PFS was 7.23 months in patients who received breast RT and 11.76 months in patients who did not receive breast RT (p=0.098, HR: 2.14, CI: 0.86-5.30). The clinical characteristics of the patients who received and did not receive breast RT previously are presented in Table 2, which reveals that both groups had similar characteristics.

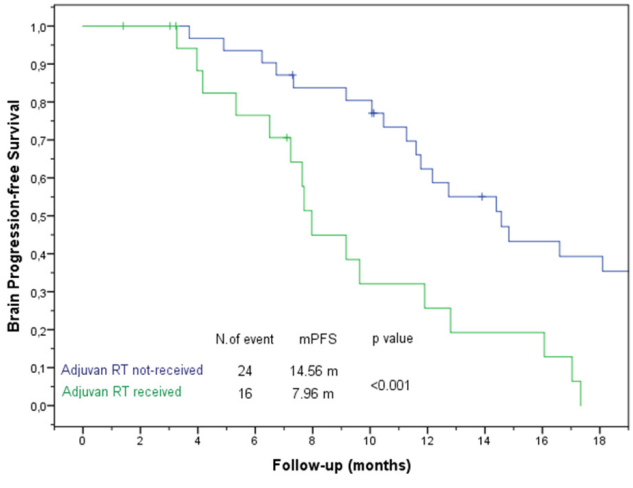

FIGURE 1: The relationship between brain PFS and whether or not breast RT was applied before.

PFS: Progression-free survival; RT: Radiotherapy

In the follow-up of patients, it was noted that 41 patients had died. The median OS time was accordingly calculated to be 25.10±4.82 months. The OS was 25.10 months for patients who did not receive adjuvant RT and 17.3 months for patients who received adjuvant RT, although the difference was not statistically significant (p=0.219).

DISCUSSION

The present study is, to the best of the author's knowledge, the first one to demonstrate that the administration of adjuvant RT diminishes the effectiveness of subsequent RT for brain metastasis.

Among all cancer types, breast cancer ranks second in terms of the development of brain metastasis, following lung cancer. The presence of brain metastasis in breast cancer patients leads to a significant reduction in the OS of patients, negatively impacting the quality of life of these patients.[10] Among the different subtypes of breast cancer, HER2-positive breast cancer is the most common subtype in which brain metastasis develops.[19] The incidence of brain metastasis is approximately 37.2% in the patients who have received multiple treatment regimens for HER2-positive breast cancer and only around 2% at the time of initial diagnosis.[15,20] Even patients with low-HER2-expression breast cancer are at an increased risk of developing brain metastasis.[21] Treatment with anti-HER2 antibodies has been demonstrated to significantly prolong the duration between the diagnosis and the development of brain metastasis. Prior to the commencement of the clinical use of trastuzumab, the duration between the diagnosis and the occurrence of brain metastasis was approximately 10 months. However, after the introduction of trastuzumab, this duration was extended to 15 months.[22] In the present study, all patients developed brain metastasis while receiving treatment with trastuzumab, and the detection occurred around 18 months after the initial diagnosis. A previous study conducted in 2011 reported achieving an iPFS of 10 months with whole-brain RT and trastuzumab treatment, while in the

TABLE 2: Baseline clinical and histological features of the patients with or without breast RT.					
			Breast RT received no (%)	Breast RT not-received no (%)	p value
Age ($\overline{X}$ ± SD)			47.35±11.60	45.00±9.68	
Time (months) from diagnosis to brain RT ($\overline{X}$ ± SD)			22.46±40.35	18.10±10.14	
Estrogen receptor expression expression		Positive	12 (60)	13 (41.9)	0.258
		Negative	8 (40)	18 (58.1)	
Stage at diagnosis		2	6 (30)	3 (9.7)	**0.020**
		3	7 (35)	5 (16.1)	
		4	7 (35)	23 (74.2)	
Prior anti-HER2 therapy	Trastuzumab	Yes	20 (100)	31 (100)	
		No	0	0	
	Pertuzumab	Yes	3 (15)	8 (25.8)	0.493
		No	17 (85)	23 (74.2)	
	TDM-1	Yes	3 (15)	4 (12.9)	1.000
		No	17 (85)	27 (87.1)	
	Lapatinib	Yes	1 (5)	1 (3.2)	1.000
		No	19 (95)	30 (96.8)	
Treatment after brain RT	Capecitabine+Lapatinib		3 (15)	6 (19.3)	0.125
	TDM-1		2 (10)	10 (32.3)	
	Trastuzumab+Cht+Pertuzumab		15 (75)	15 (48.4)	
Metastasis site (during brain RT)	Liver metastasis	Yes	5 (25)	7 (22.6)	1.000
		No	15 (75)	24 (77.4)	
	Lung metastasis	Yes	9 (45)	5 (16.1)	0.051
		No	11 (55)	26 (83.9)	
	Bone metastasis	Yes	9 (45)	17 (54.8)	0.572
		No	11 (55)	14 (45.2)	
Number of brain metastasis	Single		4 (20)	3 (9.7)	0.561
	2-5		4 (20)	8 (25.8)	
	>5		12 (20)	20 (64.5)	
RT type	Stereotactic radiosurgery		8 (40)	11 (35.5)	0.774
	Whole brain RT		12 (60)	20 (64.5)	

SD: Standard deviation; RT: Radiotherapy; Cht: Chemotherapy; HER2: Human epidermal growth factor receptor 2.

present study, this duration was approximately 12 months.[23] In an *in vivo* study on the anti-HER2-targeting treatment using Pyrotinib, it was observed that combining this treatment drug with RT significantly improved OS.[24] It was accordingly anticipated that the development of further effective anti-HER2-targeting therapies could further prolong this duration.

The susceptibility of cells to RT is influenced by the extent of DNA damage induced within the cell and the cell's capacity to activate repair mechanisms via the DNA damage response (DDR).[25] When the DDR fails to activate or the cellular DNA repair mechanisms are unable to effectively achieve DNA repair, cells enter a non-dividing state and are ultimately driven toward apoptosis via various mechanisms.[26] Cancer cells that possess an enhanced capacity for DDR tend to exhibit resistance to radiation therapy.

In head and neck cancers, for instance, the overexpression of TRIP13, which is involved in non-homologous end joining (NHEJ), and the expression of Ku80 protein reportedly promoted *in vitro* NHEJ repair and increased resistance to radiation therapy.[27,28] Activation of p53 is another critical component of the DDR mechanism, and the induction of p53 may lead to cell cycle arrest, DNA repair, or apoptosis. Clinical studies have revealed that p53 status could be a significant factor in the response to DNA-damaging agents, including RT.[29,30] Furthermore, a recent study revealed that the activation of the S100A9-RAGE-NF-κB-JunB pathway

is associated with resistance to RT in the context of brain metastasis.[18] In addition to the experimental molecular studies stated above, studies have investigated the clinical unresponsiveness to RT. Conflicting results were reported in studies comparing whole-brain RT and single high-dose RT for brain metastasis in patients with triple-negative breast cancer and lung cancer.[31-34] In the present study, no difference between WBRT and SRS was noted.

The present study identified that previous RT to the primary lesion prior to conducting RT for brain metastasis led to a significant decrease in intracranial PFS. An examination of the factors that could affect the results of the study, such as the treatments received by patients prior to and after brain RT (as presented in Table 2), and the lack of correlation between the post-RT treatments and PFS suggested that the study results are independent of the systemic treatments received.

Certain studies have suggested that the clinical course of patients diagnosed with *de novo* metastatic breast cancer is better than that of recurrent breast cancer patients.[31-34] In the present study, the proportion of *de novo* metastatic breast cancer patients was higher among the patients who did not receive breast RT, because of which a subgroup analysis had to be conducted for this subset of patients. In patients with *de novo* metastatic disease who also received breast RT, it was noted that the brain PFS was significantly shorter compared to that observed for the patients who did not receive breast RT.

Study Limitations

The limitations of the present study include its retrospective design, the fact that the molecules capable of causing RT resistance were not investigated, and the small sample size that was not sufficiently representative of the general population. In addition, the number of patients using TDM1 after RT was higher in the group that had not previously received local RT, and this could have introduced a bias in the study results and conclusions.

CONCLUSION

Breast cancer is a prevalent cause of brain metastasis, with HER2-positive brain metastasis reported as a particularly common subtype. RT is a crucial component of brain metastasis treatment. However, the present study revealed that prior RT for the primary lesion resulted in reduced efficacy of the subsequent RT for brain metastasis. This finding suggests that RT could induce molecular mutations that might contribute to the development of RT-resistant clones.

Ethics

Ethics Committee Approval: Ethical approval for the study was obtained from the Local Research Ethics Committee of the Faculty of Medicine at Hacettepe University (date: January 24, 2023, no: GO/2308).

Informed Consent: The participants provided written informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.C., S.A., Concept: H.Ç.Y., Ö.D., S.A., Design: H.Ç.Y., G.K., S.A., Data Collection or Processing: G.K., Y.E., Analysis or Interpretation: H.Ç.Y., D.C.G., M.G., Literature Search: M.G., F.Y., Writing: H.Ç.Y.

Conflict of Interest: Sercan Aksoy MD is editor-in-chief in Journal of Oncological Sciences. He had no involvement in the peer-review of this article and had no access to information regarding its peer-review. Deniz Can Güven MD is section editor in Journal of Oncological Sciences. He had no involvement in the peer-review of this article and had no access to information regarding its peer-review.

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ORIGINAL ARTICLE

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Prognostic Significance of Inflammatory and Nutritional Biomarkers in Patients with Metastatic Gastric Cancer

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ABSTRACT

Objective: Metastatic gastric cancer (mGC) is an incurable disease and a leading cause of cancer-related deaths worldwide. The prognostic significance of systemic inflammation and nutritional scores in patients with mGC has been investigated; however, optimal biomarkers for prognosis need to be identified.

Material and Methods: This single-center retrospective study included patients with synchronous or metachronous mGC. We evaluated the associations between overall survival (OS) and Eastern Cooperative Oncology Group performance status (ECOG PS), serum albumin level, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, systemic immune-inflammation index, C-reactive protein-to-albumin ratio (CAR), prognostic nutritional index, modified Glasgow prognostic score (mGPS), and inflammatory burden index.

Results: In total, 203 patients were included, with 144 (71%) males and 59 (29%) females. The median age was 59 years (range: 21-82). The median follow-up time was 13.9 months (range: 2.7-114.9 months). Univariate analysis revealed that the ECOG PS (p=0.001), body mass index (BMI) (p=0.006), serum albumin level (p=0.002), CAR (p=0.013), and mGPS (p<0.001) were significant prognostic factors for OS. In the multivariate analysis, ECOG PS ≥1 vs. 0 [hazard ratio (HR): 1.5, 95% confidence interval (CI): 1.07-2.48; p=0.018], BMI <23.20 kg/m^2 vs. ≥23.20 kg/m^2 (HR: 0.70, 95% CI: 0.53-0.98; p=0.037) and mGPS 2 vs. 0-1 (HR: 1.3, 95% CI: 1.1-1.7; p=0.001) were independent predictors of poorer OS.

Conclusion: Our findings suggested that pretreatment BMI and the mGPS may be significant prognostic biomarkers for predicting OS in patients with mGC. A low BMI and high mGPS are associated with poor survival outcomes.

Keywords: Stomach neoplasms; body mass index; nutritional status; inflammation; survival

INTRODUCTION

Gastric cancer (GC) is one of the most prevalent causes of cancer-associated mortality, with about 1 million new cases reported annually. In 2022, about 659,853 deaths occurred due to GC; its incidence and mortality rank 5th in the world.[1] GC frequently manifests as an advanced, unresectable, or metastatic disease. Advanced-stage GC is often incurable, and the main goals of systemic treatment are symptom palliation, enhancing the quality of life, and prolonging survival. Despite the median overall survival (OS) approaching about 20 months with the addition of immunotherapy and monoclonal antibodies to fluoropyrimidine and platinum-based conventional chemotherapy, the prognosis for advanced GC patients remains unfavorable.[2-4]

Cancer-associated inflammation and malnutrition are prevalent in patients with malignancies and significantly influence the progression and prognosis of tumors.[5,6] Immunologic factors affect the sensitivity of chemotherapy and may include tumor differentiation and the expression of particular genes.[7,8] Nutritional status during treatment also significantly influences the response to chemotherapy. However, accurate markers for estimating cancer response and patient prognosis before chemotherapy need to be identified for the optimal formulation of treatment strategies.

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Several studies have reported a robust link between the incidence and progression of GC and the tumor-inflammatory microenvironment.[8,9] Inflammation factors have been extensively studied as relevant prognostic indicators in patients with GC. The neutrophil-to-lymphocyte ratio (NLR), the platelet-to-lymphocyte ratio (PLR), the Glasgow prognostic score (GPS), the systemic immune-inflammation index (SII), C-reactive protein (CRP), the serum albumin level, the prognostic nutritional index (PNI), the inflammation-combined prognostic index (ICPI), and the inflammatory burden index (IBI) are associated with survival and can be used as potential prognostic indicators in patients with GC.[10-16]

Biomarkers have gained considerable attention in recent years because of their ability to perform quick, cost-effective, and convenient assessments, which enhances their clinical applicability. The usefulness and efficacy of nutritional and inflammation biomarkers in the treatment of patients with metastatic gastric cancer (mGC) require additional verification.

In this study, we investigated the prognostic significance of inflammatory and nutritional biomarkers measured by conducting blood analysis during the pretreatment period in a cohort of Turkish patients with mGC. The primary aim of conducting this study was to identify the most beneficial biomarker for prognostic evaluation.

MATERIAL AND METHODS

Patients and Data Collection

In this study, we retrospectively included 203 patients diagnosed with mGC from January 2011 to January 2023. We obtained clinicopathological data from patients' databases and medical records. Patients were selected based on the following criteria: 1) histologically confirmed GC; 2) radiologically confirmed metastatic disease; 3) measurement of serum inflammatory and nutritional markers before first-line systemic treatment; and 4) complete medical records. The exclusion criteria were as follows: absence of serum inflammatory and nutritional marker measurements, presence of other malignancies, inadequate clinical outcomes, and signs of active infection or chronic liver disease.

The patient data collected from clinical records included demographic features, Eastern Cooperative Oncology Group performance status (ECOG PS), anatomic location and histopathologic features of the primary tumor, laboratory data before first-line systemic treatment, the number and location of metastases, and the chemotherapy regimens administered. The treatment regimens and dosages used were consistent with those used in the main clinical trials.

Ethical Approval

This study was conducted according to the principles of the Declaration of Helsinki and was approved by İstanbul University-Cerrahpaşa the Local Ethics Committee for clinical trials (date: August 14, 2024; no: 1064826). Owing to the retrospective nature of this study, the requirement for informed consent was waived. As this was a retrospective study, the need for informed permission was waived.

Definitions of Inflammatory and Nutritional Biomarkers

Data on neutrophil, lymphocyte, platelet, albumin, CRP, alkaline phosphatase, and lactate dehydrogenase levels were obtained from peripheral blood tests in the database. Additionally, the PLR, NLR, SII, PNI, CRP-to-albumin ratio (CAR), IBI, body mass index (BMI), and modified GPS (mGPS) were calculated.

The values of the subsequent variables were calculated based on these results. We measured the NLR by dividing the neutrophil count by the lymphocyte count, the PLR by dividing the platelet count by the lymphocyte count, and the CAR by dividing the CRP level by the albumin level. The SII was computed as the neutrophil count×platelet count/total lymphocyte count; the IBI score was computed as the absolute value of CRP×NLR; the PNI was determined as 10×serum albumin level+0.005×total lymphocyte count; the mGPS was assessed with the serum CRP and albumin levels: CRP >10 mg/L and albumin <3.5 g/dL received a score of 2; CRP >10 mg/L or albumin ≥3.5 g/dL received a score of 1; CRP ≤10 mg/L or albumin <3.5 g/dL received a score of 1; and finally, CRP ≤10 mg/L and albumin ≥3.5 g/dL received a score of 0.

Statistical Analysis

The patients were categorized into distinct groups according to systemic inflammatory and nutritional biomarkers, including the NLR, PLR, CAR, SII, IBI, PNI, and BMI. Finally, a survival analysis was conducted on the aforementioned groups. SPSS version 26 was used to conduct the statistical assessment. We analyzed the data using conventional descriptive statistics, which included the mean, standard deviation, median, and range for continuous variables, as well as the frequency and proportion for categorical variables. To analyze categorical data, the Fisher or chi-squared test was conducted, and to analyze continuous data, a t-test was conducted to compare patient features. OS was described as the duration from the start of palliative therapy until death due to any reason or the final visit. The Kaplan-Meier method was used to estimate survival curves, and the log-rank test was conducted for comparisons. Univariate and multivariate logistic regression models were used to evaluate the factors

that contribute to OS. The Cox proportional hazards model was used to conduct a multivariate analysis to evaluate the effect of prognostic factors on OS. All results were considered to be statistically significant at $p<0.05$.

RESULTS

Characteristics of Patients

The median age of patients was 59 years (range: 21-82). There were 144 (71%) male patients and 59 (29%) female patients. The ECOG PS was 0 in 24% (n=49) of the patients, 1 in 70% (n=141), and ≥2 in 6% (n=13) of the patients. The initial demographic and clinicopathologic findings of the patients are summarized in Table 1. Among all patients, 143 (60%) had tumors in the stomach, whereas 60 (30%) had tumors in the gastroesophageal junction. According to the Lauren classification, most patients presented with diffuse-type tumors. The signet ring cell component was present in 41% of patients, and the mucinous component was present in 29% of patients. Most patients (75.4%) were human epidermal growth factor receptor type 2 (HER2)-negative, and 24.6% of patients (n=50) were HER2-positive. Synchronous metastases were present in 158 (78%) patients. The most prevalent metastatic sites were distant lymph nodes, the liver, and the peritoneum (62.1%, 43.3%, and 34.5%, respectively). According to the mGPS assessment, 33% (n=66) of patients scored 0, 49% (n=98) of patients scored 1, and 18% (n=34) of patients scored 2. The calculated nutritional and inflammation markers and scores are summarized in Table 2.

Treatment Interventions

The initial chemotherapy regimens for the patients included 5-fluorouracil plus oxaliplatin (n=99, 49.1%), 5-Fluorouracil plus cisplatin (n=85, 41.6%), Capecitabine plus oxaliplatin (n=14, 6.9%), weekly Paclitaxel (n=3, 1.4%), and 5-Fluorouracil plus Irinotecan (n=2, 1%). In the HER2-positive cohort, 94% of patients (47 of 50) received anti-HER2 treatment (Trastuzumab), whereas three patients were treated with chemotherapy combined with trastuzumab as second-line treatment. In total, 12 patients (5.8%) were administered immune checkpoint inhibitors in combination with chemotherapy as first-line therapy. Only 6 patients, four from the HER-positive group, continued first-line treatment by the evaluation cutoff date.

Second-line chemotherapy was administered to 110 patients, representing 54.4% of the cohort. The most common second-line treatment regimens included 5-Fluorouracil combined with Irinotecan (n=64, 58.1%) and weekly Paclitaxel (n=23, 20.9%). Eight patients were administered Paclitaxel in combination with Ramucirumab, while 2 patients were administered Pembrolizumab.

Survival Analyses

We found that 18 of 202 patients (8.9%) were alive at the last follow-up date. The median follow-up duration was 13.9 (range: 2.7-114.9) months. The last follow-up date was May 1, 2024. According to receiver operating characteristic analysis, no statistically significant cut-off level was found to predict survival for inflammation and nutritional markers (Figure 1). Therefore, patients were categorized into subgroups based on the median levels of the markers (NLR, PLR, PNI, SII, CAR, and IBI), and the variables affecting survival were assessed. Among the inflammatory and nutritional biomarkers, only the mGPS was significantly associated with OS. Patients with mGPS of 0-1 had better OS than those with mGPS of 2 (18.2 vs. 13.4 months, $p<0.001$). In the univariate analysis, ECOG PS (≥1 vs. 0), BMI (<23.20 kg/m^2 vs. ≥23.20 kg/m^2), serum albumin level (<3.5 g/dL vs. ≥3.5 g/dL), and mGPS (2 vs. 0-1) were associated with worse OS. The Kaplan-Meier curves of OS are shown in Figure 2. The multivariate analysis indicated that ECOG PS ≥1 vs. 0 [hazard ratio (HR: 1.5, 95% confidence interval (CI): 1.07-2.48; p=0.018], BMI <23.20 kg/m^2 vs. ≥23.20 kg/m^2 (HR: 0.70, 95% CI: 0.53-0.98; p=0.037), and mGPS 2 vs. 0-1 (HR: 1.3, 95% CI: 1.1-1.7; p=0.001) were independently associated with worse OS. The univariate and multivariate analyses are summarized in Table 3.

DISCUSSION

In this study, we assessed the effect of systemic inflammatory and nutritional factors, including the NLR, PLR, SII, CAR, IBI, mGPS, BMI, and PNI, on survival outcomes in patients

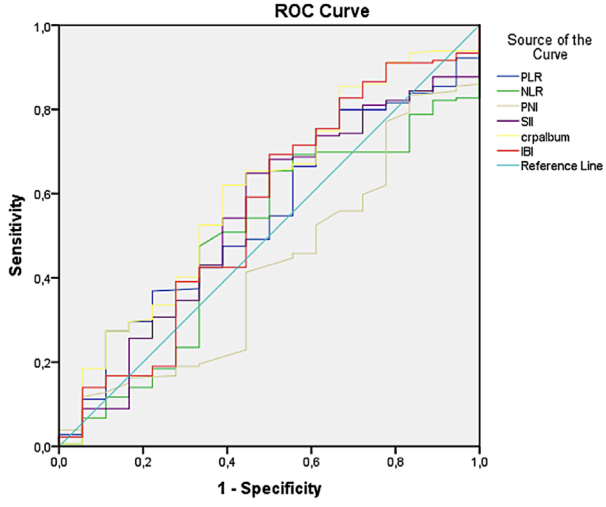

FIGURE 1: Receiver operating characteristic analysis for the inflammation and nutrition-based markers.

ROC: Receiver operating characteristic; PLR: Platelet-to-lymphocyte ratio; NLR: Neutrophil-to-lymphocyte ratio; PNI: Prognostic nutritional index; SII: Systemic immune-inflammation index; CAR: C-reactive protein-to-albumin ratio; IBI: Inflammatory burden index.

diagnosed with mGC. Our findings indicated that the ECOG PS, serum albumin level, BMI, CAR, and mGPS were significantly associated with OS in patients with mGC. Moreover, the results of our analysis revealed that the ECOG PS, BMI, and mGPS were significantly correlated with OS, independent of other predictive factors.

The systemic inflammatory response affects oncological outcomes in cancer patients. Additionally, the nutritional status of patients also plays a significant role in influencing tumor progression.[17] The relationship among systemic inflammation, nutritional status, and cancer patient prognosis involves complex mechanisms and is not fully understood. Several studies have investigated the effect of inflammation and nutritional markers on survival and prognosis in patients diagnosed with GC.[18-21] A meta-analysis involving 18,348 patients demonstrated that an increase in CRP levels, NLR, and GPS/mGPS is correlated with worse survival outcomes in GC patients.[18] Another meta-analysis involving 1,336 patients with advanced GC undergoing immunotherapy revealed that elevated NLR and PLR were correlated with shorter OS.[19] A comprehensive analysis of 14,403 patients across 25 studies indicated that a low preoperative PNI might be associated with a significant occurrence of postoperative complications and an unfavorable prognosis in patients with GC.[20] A retrospective study conducted by Sugiyama et al.[21] showed that active nutritional support can improve the prognosis of patients with mGC undergoing chemotherapy.

Several studies have shown that low albumin levels are significantly correlated with reduced survival rates in GC patients.[22,23] GC patients frequently exhibit poor nutritional status due to tumor infiltration of the stomach or pyloric stenosis, resulting in low serum albumin levels. Additionally,

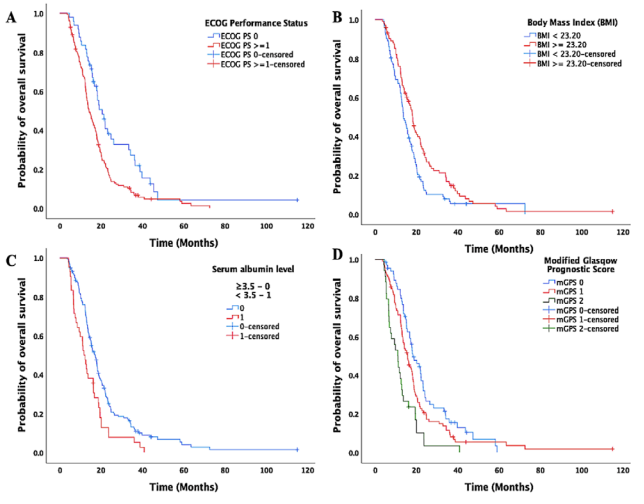

FIGURE 2: Kaplan-Meier: *Eastern Cooperative Oncology Group performance status*.

A; Body mass index B; Serum albumin levels C; and modified Glasgow Prognostic Score (mGPS) D; ECOG: Eastern Cooperative Oncology Group; PS: Performance status; OS: Overall survival; BMI: Body mass index; mGPS: Modified Glasgow prognostic score.

TABLE 1: Baseline demographic and clinicopathologic findings.

Variables (n=203)		n (%)
Age (years)	Median	59 (range 21-82)
	<65	135 (67)
	≥65	68 (33)
Gender	Female	59 (29)
	Male	144 (71)
ECOG PS	PS 0	49 (24)
	PS 1	141(70)
	PS≥2	13(6)
BMI (kg/m^2) (median)		23.20 (range: 14.4-37.6)
Location	Gastroesophageal junction	60 (30)
	Stomach	143 (70)
Lauren classification	Diffuse	112 (55)
	Intestinal	56 (28)
	Unknown	35 (17)
Signet ring cell component		84 (41)
Mucinous component		58 (29)
Microsatellite instabilty-high		2 (1)
HER-2 status	Negative	142 (70)
	Positive	50 (25)
	Unknown	11 (5)
CEA	>ULN	103 (56)
	≤ULN	80 (44)
CA 19-9	>ULN	102 (56)
	≤ULN	81 (44)
De novo metastastasis		158 (78)
Metastatic site, n (%)	Liver	88 (43)
	Peritoneum	70 (35)
	Lung	29 (14)
	Distant lymp nodes	126 (62)
	Bone	23 (11)
	Others	14 (7)
Status	Alive	18 (9)
	Exitus	185 (91)
OS (months)	Median	15.9 (95% CI: 13.7-18.1)

ECOG PS: Eastern Cooperative Oncology Group performance status; BMI: Body mass index; HER2: Human epidermal growth factor receptor type 2; CEA: Carcinoembryonic antigen; CA 19-9: Carbohydrate antigen 19-9; ULN: Upper limit of normal; OS: Overall survival.

hypoalbuminemia can appear because of an ongoing systemic inflammatory response, which can negatively affect cancer-specific survival in patients with GC. Elevated CRP levels indicate increased systemic inflammation; consequently, the CAR can be used as a marker for systemic inflammation and nutritional status. A meta-analysis including 3,102 patients from 8 observational studies showed that a high pretreatment CAR was significantly correlated with reduced survival rates ($p<0.001$) for patients with GC.[24] Similar to the findings in other studies, our findings indicated that low albumin levels and high CAR significantly correlate with poorer survival outcomes.

The ECOG PS is a basic tool for determining the physical condition of patients and provides a generally accepted prognostic factor for predicting survival outcomes in cancer patients.[25] A study by Fanotto et al.[26] included 704 mGC patients and reported that patients with an ECOG PS of 2 had significantly shorter progression-free survival and OS than those with PS of 1 and 0. Another study investigating patients with mGC reported that an ECOG PS ≥2 was an independent poor prognostic factor for predicting OS.[27] The results of our study also indicated that patients with an ECOG PS of 0 had significantly better OS than those with an ECOG PS of ≥1.

Patients with mGC often exhibit a generalized loss of skeletal muscle mass and strength, which is frequently attributed to nutritional deficiencies caused by tumor localization and tumor-related inflammation. A meta-analysis conducted by Borggreve et al.[28] that included 4,887 patients with GC showed that patients with low muscle mass had significantly higher rates of postoperative complications, severe postoperative complications, and overall mortality. BMI can serve as a reliable indicator for assessing the nutritional status of cancer patients. The relationship between BMI and survival outcomes in patients with GC is under investigation. Feng et al.[29] examined the relationship between BMI and outcomes in 1,210 patients treated with D2 gastrectomy and revealed that a lower BMI was associated with a reduced incidence of postoperative fever and poorer survival outcomes. Another study evaluated 7,765 patients with GC who underwent surgery at a single institution. Patients with a BMI of 23-30 kg/m^2 before gastrectomy showed better OS and disease-specific survival rates than those with a BMI of <23 kg/m^2.[30] This study also revealed a significant relationship between low BMI (<23.20 kg/m^2) and poor OS in patients with mGC.

TABLE 2: Results of systemic inflammatory and nutritional marker analysis in the cohort.

Variables		Median (range)
LDH		195 (13-2318)
ALP		94 (26-2271)
CRP		11 (0.1-227)
Albumin		4.0 (2.3-5.1)
NLR		2.98 (0.21-65)
PLR		207.2 (45-3710)
PNI		47.5 (13-63)
SII		105.01 (2.66-2411.5)
CAR		2.75 (0.02-81.07)
IBI		34.45 (0.13-2814)
mGPS, n (%) (n=198)	0	66 (33)
	1	98 (49)
	2	34 (18)

LDH: Lactate dehydrogenase; ALP: Alkaline phosphatase; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio (PLR); PNI: Prognostic nutritional index; SII: Systemic immune-inflammation index; CAR: C-reactive protein-to-albumin ratio; IBI: Inflammatory burden index; mGPS: Modified Glasgow prognostic score.

The mGPS is a well-documented inflammation-based prognostic assessment of survival for different types of cancer, including GC.[27,31-34] In previous studies, the predictive value of the mGPS in GC has been investigated mostly in patients with early-stage and locally advanced-stage disease. Zhang et al.[35] investigated 488 GC patients who underwent curative surgery and had normal preoperative serum levels of Carcinoembryonic antigen and Carbohydrate antigen 19-9 to assess the prognostic value of the mGPS for OS. They found significant differences among patients with mGPS of 0, 1, and 2 ($p<0.001$), indicating that a higher mortality rate was associated with a higher mGPS. The results of a meta-analysis including 3,206 GC patients across seven studies showed that OS was significantly lower in patients with mGPS of 1 and 2 than in patients with a score of 0 ($p<0.01$).[36] Demirelli et al.[27] evaluated the relationship between nutritional/inflammatory markers and survival in patients with mGC and revealed that mGPS, PNI, and ECOG scores were independent indicators of shorter survival. Similarly, the results of this study indicated that the mGPS is an independent negative predictive biomarker affecting OS in mGC patients.

Study Limitations

The results obtained in this single-center, real-world study should be interpreted with caution as this study had several limitations. The retrospective collection of data from clinical databases can reveal potential selection biases and influencing factors that may affect the interpretation of the results. Second, we could not control for certain potential cofactors influencing inflammation-related and/or nutritional markers. The incorporation of these parameters in future prospective studies may facilitate a more comprehensive evaluation of the prognostic and predictive importance of inflammatory and nutritional biomarkers in mGC patients.

TABLE 3: Univariate and multivariate analyses of overall survival in advanced gastric cancer patients.

		Median OS (95% CI)	Univariate analysis		Multivariate analysis	
			HR (95% CI)	p value	HR (95% CI)	p value
Gender	Female	17.1 (13.8-20.5)	1.2 (0.9-1.7)	0.15		
	Male	15.6 (13.1-18.0)				
Age	<65	15.5 (13.4-17.6)	0.8 (0.6-1.2)	0.44		
	≥65	16.6 (12.1-21.2)				
ECOG PS	PS 0	20.4 (16.0-24.8)	1.7 (1.2-2.5)	**0.001**	1.5 (1.07-2.48)	**0.018**
	PS ≥1	14.0 (12.1-15.9)				
BMI, kg/m^2	≥23.20	18.1 (16.1-20.0)	0.66 (0.4-0.89)	**0.006**	0.70 (0.53-0.98)	**0.037**
	<23.20	13.7 (11.9-15.4)				
Serum albumin	≥3.5 g/dL	17.1 (15.01-19.1)	1.7 (1.2-2.4)	**0.002**	0.89 (0.3-1.8)	0.76
	<3.5 g/dL	11.6 (9.6-13.6)				
NLR	<2.98	16.2 (13.8-18.6)	1.1 (0.8-1.5)	0.42		
	≥2.98	14.3 (11.4-17.1)				
PLR	<207.2	17.5 (15.0-19.5)	1.2 (0.9-1.6)	0.17		
	≥207.2	13.9 (11.5-16.3)				
PNI	≥47.5	17.7 (15.7-19.8)	0.7 (0.5-1.05)	0.16		
	<47.5	13.9 (11.5-16.3)				
SII	<105.01	16.1 (14.2-18.8)	1.1 (0.8-1.5)	0.39		
	≥105.01	14.0 (11.5-16.5)				
CAR	<2.75	17.8 (16.2-19.5)	1.4 (1.08-1.9)	**0.013**	1.2 (0.8-1.6)	0.26
	≥2.75	13.0 (11.6-14.3)				
IBI	<34.45	17.1 (15.4-18.8)	1.2 (0.9-1.6)	0.13		
	≥34.45	13.4 (10.8-16.1)				
mGPS	0-1	18.2 (13.8-22.5)	1.4 (1.2-1.7)	**<0.001**	1.3 (1.1-1.7)	**0.001**
	2	13.4 (11.2-15.6)				

ECOG: Eastern Cooperative Oncology Group; PS: performance status; OS: Overall survival; CRP: C-reactive protein; NLR: Neutrophil-to-lymphocyte ratio; PLR: Platelet-to-lymphocyte ratio; PNI: Prognostic nutritional index; SII: Systemic immune-inflammation index; CAR: C-reactive protein-to-albumin ratio; IBI: Inflammatory burden index; mGPS: Modified Glasgow prognostic score.

CONCLUSION

The optimal inflammatory and nutritional scoring system for assessing the prognosis of patients with mGC is under investigation. The primary objective of this study was to identify the best biomarker for predicting the prognosis of patients with mGC, and our findings suggested that BMI and mGPS may be the most effective biomarkers for predicting survival outcomes.

Ethics

Ethics Committee Approval: This study was conducted according to the principles of the Declaration of Helsinki and was approved by İstanbul University-Cerrahpaşa the Local Ethics Committee for clinical trials (date: August 14, 2024; no: 1064826).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Idea/Concept: M.G., Ö.A.; Design: M.G., M.Gü., Control/Supervision: Ö.A., N.S.D., Data Collection and/or Processing: M.C.F., S.S., Analysis and/or Interpretation: M.G., Ö.A., Literature Review: G.A.Ş., M.Gü., Writing the Article: M.G., Critical Review: Ö.A., References and Fundings: M.G., N.S.D.

Conflict of Interest: No conflict of interest was declared by the authors.

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ORIGINAL ARTICLE

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Relationship between the Release of Interleukin 12 and the Expression of PCNA, Cyclin A2, and CDK2 in Lung Cancer Cells

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ABSTRACT

Objective: Mesenchymal stem cell (MSC)-based IL-12 release therapy may be less toxic than direct administration of IL-2. We investigated the release of interleukin (IL)-12 in MSCs and A549 cocultures and identified the time points at which IL-12 is secreted. We also examined changes in the expression of proliferative genes, such as proliferating cell nuclear antigen (PCNA), cyclin A2, and cyclin-dependent kinase 2 (CDK2).

Material and Methods: This study was conducted by culturing A549 cells, MSCs, and MSC-A549 cells at 3 different time points. The release of IL-12p70 from cells in the collected culture media was determined by Enzyme-Linked ImmunoSorbent Assay. The expression of the *PCNA*, cyclin *A2*, and *CDK2* genes involved in the cell cycle was determined quantitatively by reverse transcriptase polymerase chain reaction.

Results: The expression of PCNA was the lowest, and the expression of the cyclin *A2* and *CDK2* genes was the highest in cocultured A549 cells, whereas the expression of the IL-12 protein was the highest at the 12th h of coculture. The expression levels of the cyclin *A2* and *CDK2* genes decreased in cocultured A549 cells at the 24th h in the presence of released IL-12. However, in A549 cells cultured alone, when the IL-12 level was the highest at the 24th h, the cyclin A2 and CDK2 expression levels were high compared to those in the control group.

Conclusion: IL-12 protein affects cell cycle regulatory genes, slows down cell proliferation, and may affect the prognosis of patients with this disease.

Keywords: Lung neoplasms; mesenchymal stem cells; interleukin-12; cell cycle proteins

INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide. Although lung neoplasms are a heterogeneous group of more than 50 histomorphological subtypes, they are generally classified as non-small cell lung carcinoma (NSCLC) or small cell lung carcinoma (SCLC) because limited treatment options do not require considerable morphological subclassification. While NSCLCs account for 80-85% of all lung cancers, their most common histological subtype is adenocarcinoma, with a rate of about 40%.[1,2]

Stem cells are undifferentiated and can regenerate, differentiate, form clones derived from a single cell, and ensure the continuity and regeneration of tissues.[3,4] They are responsible for the regeneration of cells that are damaged in tissues due to physiological or pathological processes, the production of soluble factors necessary for cell survival and reproduction, and the regulation of the immune response.[5,6] Among adult stem cells, the most commonly used cell types in stem cell therapy are mesenchymal stem cells (MSCs) and hematopoietic stem cells.[3]

MSCs originate from the stroma and can be obtained from many tissues in the organism. Compared to bone marrow-derived stem cells, adipose-derived MSCs have greater isolation efficiency and proliferation capacity.[7] MSCs can

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migrate to ischemic areas and primary or metastatic tumor sites, and this migration makes them a tool for targeted therapy.[8] The use of genetically modified MSCs in targeted treatments ensures that anticancer agents are continuously distributed.

Cytokines act as cell signal proteins in intercellular communication. They bind to their receptors on the target cell after they are released. The activation resulting from this binding initiates intracellular signaling, which changes cell functions.[9] Cytokines are not constantly released; rather, they are usually produced and released in response to stimuli. They are involved in adaptive inflammatory host defense, cell growth, differentiation, and death, angiogenesis, maintenance of homeostasis, and regulation of immune responses.[10] Among these cytokines, interleukin 12 (IL-12) suppresses the growth of cancer cells by stimulating immune system cells via the production of antiangiogenic factors in cancerous tissue.[11] IL-12 plays an important role in the production of interferon gamma, the differentiation of helper T-cells, the proliferation of activated T and natural killer cells, and the stimulation of anticancer responses.[12,13] Cytokine-based immunotherapy can effectively treat many malignancies. As IL-12 causes tumor cell death, it can be considered a strong candidate for immunotherapy-based interventions. However, systemic administration of IL-12 is highly toxic; therefore, alternative methods, such as IL-12 delivery or release from cells, are needed.[14] MSC-mediated IL-12 release may be less toxic than direct administration of IL-2.

Many studies have reported that the abnormal activity of cell cycle proteins, such as cyclin-dependent kinase 2 (CDK2) and cyclin A2, which play important roles in the progression of the cell cycle, is closely related to cancer.[15-17] Abnormal activity of CDK2 causes proliferation in prostate cancer and NSCLC, as well as the transformation of mammary epithelial cells into cancer cells.[18] The expression of cyclin A2 is associated with cellular proliferation and is an indicator of poor prognosis.[19] Cyclin A2 deficiency causes cells to display characteristics similar to those of cancer stem cells.[20] Additionally, studies have shown shortened survival in patients with NSCLC positive for cyclin A.[21] Proliferating cell nuclear antigen (PCNA) acts as a bridge between CDK2 and its substrates by binding with the cyclin A-CDK2 complex during the cell cycle.[22] A high level of expression of PCNA in NSCLC is associated with a poor prognosis, and patients with PCNA-positive carcinoma have a shorter survival time than patients with PCNA-negative carcinoma.[23,24] Studies on the effects of IL-12 on cyclin A2, CDK2, and PCNA are rare.

In this study, MSCs and A549 lung cancer cells were cultured alone and together in vitro to analyze spontaneous IL-12 release from cells into the medium and monitor changes in the expression of cell cycle genes, including PCNA, cyclin A2, and CDK2, in cultured cells under these conditions. The data related to the effects of IL-12 on cyclin A2, CDK2, and PCNA are limited.

MATERIAL AND METHODS

Cell Culture

Adipose-derived MSCs (obtained from Acıbadem University) were added to DMEM F12 (Dulbecco's modified Eagle's medium/nutrient mixture F-12; Gibco, Carlsbad, CA, US) supplemented with 1% penicillin/streptomycin (Gibco, Carlsbad, CA, US) and 25% fetal bovine serum (FBS; Gibco, Carlsbad, CA, US). A549 cancer cells (obtained from Yeditepe University) were cultured in DMEM F12 medium containing 1% penicillin/streptomycin and 10% FBS. The cells were grown in a CO_2 incubator with 95% O_2 and 5% CO_2 at 37 °C overnight. The next day, the cells were seeded in a serum-free medium. Cell culture experiments were performed by establishing 3 independent cell groups: A549 cell culture alone, MSC culture alone, and MSC-A549 coculture. The Transwell system was used to assess the paracrine effects of the combination of MSCs and A549 cells. This system consisted of 2 chambers separated by a semipermeable membrane with a pore size of 0.4 µm (FALCON, Tewksbury, MA, USA). A549 cells (3×10^5 cells/well in six-well plates) were cultured in the upper chamber, and MSCs (3×10^5 cells/well in six-well plates) were cultured in the lower chamber of the Transwell inserts.

Preparation of the Conditioned Medium

After 2, 12, and 24 h, the medium was aspirated from the Transwell insert systems and centrifuged at 1000×g for 10 min. The supernatant obtained was filtered through 0.2 µm pores (Millipore Corporation SCILOGEX, Bedford, MA, USA) and stored at -80 °C for Enzyme-Linked ImmunoSorbent Assay (ELISA).

Culture Supernatant Analysis by ELISA

After 2, 12, and 24 h of A549 alone, MSC alone, and A549+MSC coculture, the supernatants were collected and ELISA was performed with these media to measure the level of the cytokine IL-12p70 secreted by A549 cells and MSCs. These measurements were made using an ELISA kit (Picokine, Valley Ave Pleasanton, CA, USA) following the manufacturer's protocols, and the absorbance of each well at 450 nm was measured using a microplate reader. The IL-12 protein concentration was calculated based on standard curves.

Detection of Change in Gene Expression Levels with QRT-PCR Application

For each experimental design, 3×10^5 cells were plated in six-well plates. The following day, the medium was aspirated, the cells were washed with phosphate saline buffer (PBS, Gibco, Carlsbad, CA, US), and FBS-free medium was added to the wells. The medium was aspirated, the cells were washed with phosphate buffer, and 0.05% trypsin-EDTA (Gibco, Carlsbad, CA, US) was added to the A549 cancer cells. Next, 0.25% trypsin-EDTA was added to the MSCs, which were incubated for 2, 12, or 24 h in a serum-free medium for RNA isolation. RNA was isolated using a NucleoSpin (Macherey Nagel, Bethlehem, PA, USA) RNA isolation kit following the manufacturer's protocol. The concentration of RNA was measured using a NanoDrop system (Multiskan Go Microplate Spectrophotometer, Thermo Fisher Scientific, Ratastie, P.O., Finland).

The cDNA synthesis kit (ProtoScript, BioLabs, New England) was used to synthesize cDNA from RNA, following the manufacturer's protocol. The amount of RNA used for cDNA synthesis was fixed to 1 µg. Then, cDNA was synthesized using a Bio-Rad T100 Thermal Cycler (Dubai, PO, United Arab Emirates).

The expression levels of the target genes were quantitatively determined by the delta delta Ct ($2^{-\Delta\Delta Ct}$) method, with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) used as a reference gene. Quantitative gene expression RT-PCR was performed using a Bio-Rad Real-Time System (Bio-Rad, Dubai, PO, United Arab Emirates). The NCBI, Primary Blast, and Primer3 programs were used for primer design and were purchased from the Sentromer Company. The primer list is provided in Table 1.

All experiments were repeated twice biologically and twice technically.

Statistical Analysis

All data were analyzed using GraphPad Prism Statistics version 9. The mRNA expression levels of the genes and the mean and standard deviation of the ELISA results were determined. Two-way analysis of variance was performed to determine differences in the data between the 2 groups. All differences were considered to be statistically significant at $p<0.01$.

RESULTS

Assessment of the IL-12P70 Level by ELISA

The media obtained from the cocultures were used to measure cytokine levels by ELISA. The presence and amount of IL-12p70 in the media collected from the 3 different cell culture groups were analyzed after they were cultured for 2, 12, and 24 h. The data obtained in the second hour were used as a control for each cell culture group. The results obtained at the 12^{th} h and 24^{th} h were compared to those of the control group.

As shown in Figure 1, the measured levels of IL-12p70 (Table 2) in the culture media following 12 h and 24 h of incubation were significantly different from those of the control time point for each culture group. The highest IL-12p70 protein levels in the coculture medium were obtained after 12 h ($p<0.0001$). However, when the cells were evaluated separately without coculturing, the highest protein content was detected in the culture media of A549 cells and MSCs after 24 h of incubation, and the difference was statistically significant ($p<0.0001$).

Determination of mRNA Levels of Genes by qRT-PCR

At the end of 12 h and 24 h of culture, the MSCs and A549 cells were cultured alone, and the cocultured MSCs and A549 cells were harvested. Then, RNA was isolated, and the expression levels of the PCNA, cyclin A2, and CDK2 genes were determined. MSCs were used as a healthy control group

TABLE 1: Primers for qRT- PCR.

Primers	Sequences
Proliferatin cell nuclear Antige11 (PCNA)	Fonvard-S'-CCAGAGCTCTTCCCTTACGC-3' Reverse- 5'-TCTAGCTGGTTTCGGCTTCA -3'
Cydin A2	Fonvard- S'-AAGACTGGCATCCAAGAAGTTT-3' Revers&- 5'-TGGTTTTACTCTCATCTTGCCAC-3'
Cycline dependent kinase 2 (CDK2)	Fomard- S'-GGATGCCTCTGCTCTCACTG-3' Revers&- 5'-GAGGACCCGATGAGAATGGC -3'
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	Fonvard- S'-CGAGATCCCTCCAAAATCAA-3 ' Reverse- S'-TTCACACCCATGACG AACAT-3'

qRT-PCR: Real-time polymerase chain reaction.

to determine target gene expression levels between each cell group, and expression at 2 h of incubation was used as a control for time-dependent comparisons of target gene expression in each cell group separately.

As shown in Figure 2, after 12 h of incubation, when the IL-12p70 level was the highest, the expression of PCNA, a cell cycle marker, decreased 0.683-fold in A549 cells cultured alone and 0.134-fold in cocultured A549 cells compared to the control cells ($p<0.0001$). In contrast, after 24 h of incubation, when the level of the IL-12 protein started decreasing, the maximum level of PCNA was detected in cocultured A549 cells, with a 1.196-fold increase ($p=0.0008$). At the end of 12 h, the level of expression of the PCNA gene in cocultured A549 cells was determined to be 0.225-fold lower than that in A549 cells cultured alone, and the difference was significant ($p=0.0047$). When IL-12 protein levels in the medium were high after 24 h of incubation, the protein expression in cocultured A549 cells was 1.785 times greater than that in A549 cells alone, and the difference was significant ($p=0.0093$). The expression levels of PCNA are shown in Table 3.

As shown in Figure 3, the level of cyclin A2, another cell cycle promoter, increased 12.88-fold in cocultured A549 cells after 12 h but 7.46-fold after 24 h of incubation ($p<0.0001$). After 12 and 24 h of incubation, A549 cells cultured alone presented a 2.86-fold and 13.34-fold increase in mRNA expression levels, respectively, compared to those at the control time point ($p<0.0001$). At the end of 12 h, the expression level of the

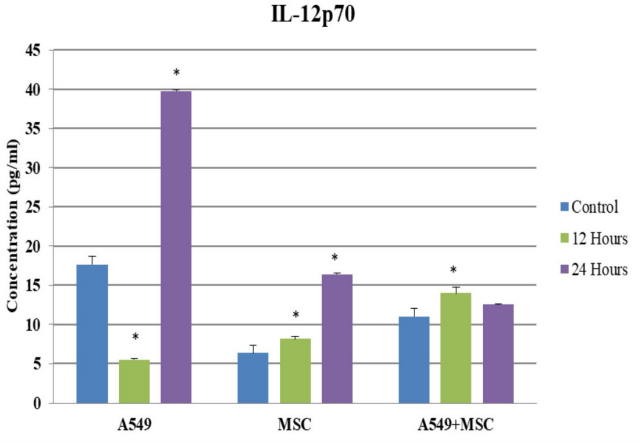

FIGURE 1: IL-12p70 results obtained by ELISA. *IL-12p70 levels are significantly different compared to those in the control group ($p<0.01$).

IL: Interleukin; MSC: Mesenchymal stem cell

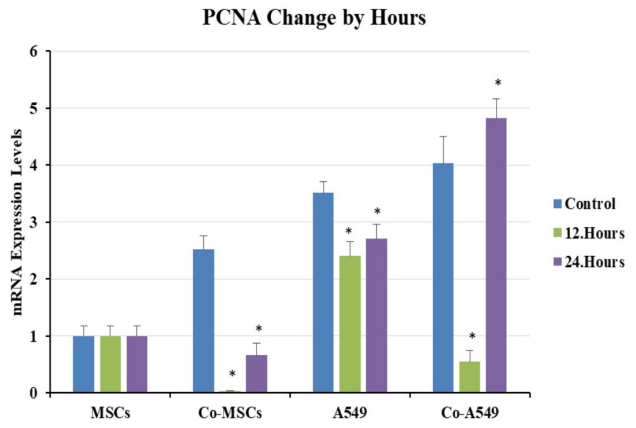

FIGURE 2: PCNA mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in MSCs alone, A549 cells alone, and cocultured cell groups over time ($p<0.01$).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction; PCNA: Proliferating cell nuclear antigen

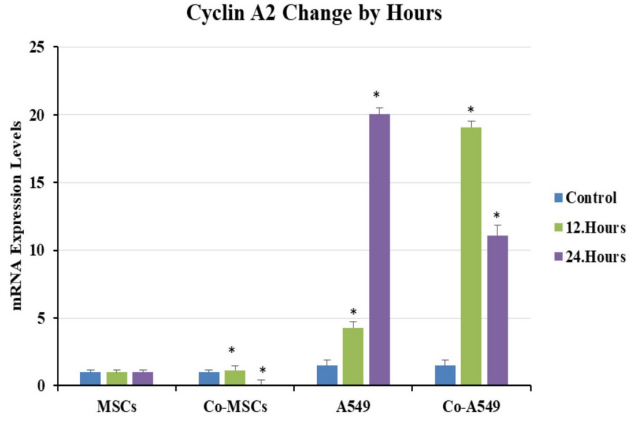

FIGURE 3: Cyclin A2 mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in MSCs alone, A549 cells alone, and cocultured cell groups over time ($p<0.01$).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction

TABLE 2: IL-12p70 protein levels obtained by ELISA at the selected time points.

Groups	IL-12p70 levels after 2 b Mean ± SD	IL-12p70 levels after 12 b Mean ± SD	IL-12p70 levels after 24 b Mean ± SD
AS49 cells	17.65±3.42 pg/mL	5.525±1.18 pg/mL	39.775±1.71 pg/mL
MSC	6.40±0.26 pg/mL	8.150±3.16 pg/mL	16.40±2.10 pg/mL
A549+MSC	11.025±3.03 pg/mL	14.025±7.77 pg/mL	12.525±0.92 pg/mL

IL: Interleukin; ELISA: Enzyme-linked ImmunoSorbent assay; MSC: Mesenchymal stem cell, SD: Standard deviation.

cyclin A2 gene in cocultured A549 cells was upregulated 4.44-fold compared to that in A549 cells cultured alone (p=0.0002). At the end of 24 h, the expression level in cocultured A549 cells was downregulated 0.55-fold compared to that in A549 cells cultured alone (p=0.0001). The expression levels of cyclin A2 are shown in Table 3.

As shown in Figure 4, the mRNA levels of the *CDK2* gene in cocultured A549 cells increased 1.06-fold (p=0.5273) after 12 h of incubation and decreased 0.69-fold after 24 h of incubation (p=0.0010). A549 cells cultured alone increased by 1.25-fold (p=0.0774) and 1.71-fold after 12 and 24 h of culture, respectively (p<0.0001). At the end of 12 h, the CDK2 expression level of the cocultured A549 cells was upregulated 1.46-fold compared to that of the A549 cells cultured alone (p=0.0085), and the results were statistically significant. At the end of 24 h, the expression level of the cocultured A549 cells was downregulated 0.69-fold compared to that of the A549 cells cultured alone, and the results were not statistically significant (p=0.1541). The expression levels of CDK2 are shown in Table 3.

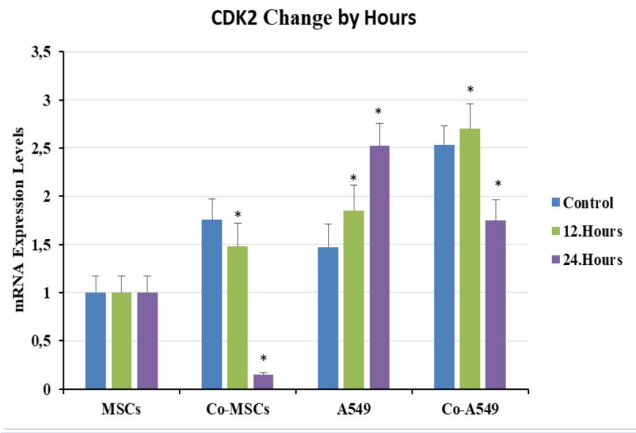

FIGURE 4: CDK2 mRNA expression results obtained by qRT-PCR. Changes in the expression levels of the mRNAs in the MSCs alone, A549 cell alone, and cocultured cell groups over time (p<0.01).

MSC: Mesenchymal stem cell; qRT-PCR: Real-time polymerase chain reaction; CDK2: Cyclin-dependent kinase 2

DISCUSSION

In this study, the IL-12p70 levels significantly changed over time when the cells were cocultured, and the effects of these changes on gene expression were significant. The results revealed that IL-12p70 levels varied over time and that their effects on cellular processes were significant. IL-12p70 content reached its maximum level during 12 h of incubation, and the decrease in the expression of PCNA, a cell cycle marker, in A549 cells during this period supported the suppressive effect of IL-12 on proliferation. In contrast, the decrease in IL-12p70 levels at the end of 24 h, followed by an increase in the expression of PCNA, suggested that the effect of IL-12 may change over time and that this change may be related to the dynamics of cellular responses. These findings indicated that IL-12 may regulate the cell cycle through direct or indirect effects as an immune modulator. The results of gene expression analyses also revealed the effect of IL-12p70 levels on the expression of cell cycle regulatory genes such as cyclin A2 and CDK2. The significant increase in cyclin A2 levels in the coculture medium at the end of 12 h suggested that IL-12 can stimulate the expression of cell cycle-related genes. However, the decrease in cyclin A2 expression observed at 24 h of incubation suggested that the effect of IL-12 on the cell cycle varies with time. Similarly, the increase in CDK2 expression peaked at 12 h and decreased at 24 h, suggesting

TABLE 3: mRNA expression levels and fold change in expressions of the cultured cells at the selected time points.

	Control	12 hours			24 hours		
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	Fold change		$\bar{X}$ ± SD	Fold change	
MSCs_PCNA	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCs_PCNA	2.52±0.23	0.03±0.01	↓0.01		0.66±0.22	↓0.26	
A549_PCNA	3.51±0.2	2.4±0.26	↓0.68	↓0.255	27.±0.26	↓0.88	↑1.785
Co-A549_PCNA	4.03±0.46	0.54±0.20	↓0.13		4.82±0.34	↓1.196	
MSCS_Cyclin A2	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCS_Cyclin A2	1±0.17	1.15±0.30	↑1.15		0.34±0.34	↓0.34	
A549_Cyclin A2	1.5±0.43	4.29±0.43	↑2.86	↑4.44	20.01±0.46	↑13.34	↓0.55
Co-A549_Cyclin A2	1.49±0.43	19.08±0.47	↑12.88		11.09±0.74	↑7.46	
MSCs_CDK2	1±0.17	1±0.17	1		1±0.17	1	
Co-MSCs_CDK2	1.76±0.22	1.48±0.24	↓0.84		0.15±0.02	↓0.08	
A549_CDK2	1.47±0.23	1.85±0.26	↑1.25	↑1.46	2.52±0.23	↑1.71	↓0.69
Co-A549_CDK2	2.53±0.20	2.7±0.26	↑1.06		1.75±0.22	↓0.69	

↓ : Fold decrease in expression, ↑: Fold increase in expression; SD: Standard deviation; Co: Bicultural.

that IL-12 can regulate not only proliferation but also different stages of the cell cycle.

Stem cells are undifferentiated cells that can renew themselves and differentiate into different types of cells.[25] Stem cells obtained from adipose tissue are the focus of attention in cell therapy, as they can be isolated from adipose tissue via a less invasive procedure than other sources of stem cells.[26,27] In cancer treatment, stem cells increase vascularization, suppress immune reactions, and ultimately promote the growth and invasion of the mass. On the other hand, they are used as vectors in targeted therapy and exhibit antitumor effects. Studies have shown that human MSCs decrease the growth of cancer when they are administered to Kaposi's sarcoma model mice. The communication between MSCs and cancer cells is facilitated by the cytokines secreted from them, and the presence of cytokines in the environment reduces the reliability of stem cell treatments.[28-31] The main finding of this study is that stem cell-produced IL-12 affects cell cycle regulatory genes, and further studies may reveal its effects on cell proliferation and the prognosis of this disease.

Cytokines are signal proteins involved in communication between cells. After being released, they are activated by binding to their receptors on the target cell, triggering signal transduction in the cell.[10] IL-12 is a powerful anticancer cytokine that suppresses the growth of cancerous tissue by suppressing angiogenetic factors such as Vascular endothelial growth factor, stimulating apoptosis, and consequently increasing the activation of p53.[11,32]

IL-12 consists of the subunits p40 and p35. The p40 subunit is present in the cell as monomers and homodimers under normal conditions, and in the presence of p35, it creates p70 through a reduction in both forms. Some studies have shown that the half-life of p35 (one of the subunits) is 2 h, and in the presence of p40, this period may extend up to 4 h. The half-life of the p40 subunit is more than 4 h, and it is not affected by the presence of p35. However, for IL-12p70 to be released, it must be activated by combining with its receptors on the target cell. Therefore, optimal production occurs due to the balanced combination of the two subunits of the IL-12 protein and its receptors.[33,34] The half-life of IL-12 produced via recombinant DNA technology is 12 h. The desire to extend this period has contributed to the use and development of other methods, such as viral vectors, exosomes, and gene therapy, in cell treatments.[35,36]

The ELISA results revealed that the maximum level of IL-12 was released at the 24th h in the A549 and MSC groups cultured alone. However, at the end of the 12th h in A549 cells, IL-12 release was minimal compared to that of the control. This occurred probably because of an imbalance in the production of IL-12 subunits or receptors. On the other hand, maximum cellular secretion of endogenously produced IL-12 protein in the medium was first detected at 12 h in the MSC-A549 coculture. This occurred probably because the interaction of 2 different cell lines in the environment increased the expression of IL-12 receptors and subunits, and therefore, the release of IL-12.

The PCNA protein is responsible for DNA synthesis, cell cycle control, and DNA damage repair by wrapping chromatin. An increase in the expression of cell cycle genes is an indicator of cell proliferation, and this is considered to be an indicator of poor prognosis for diseases with high cell proliferation, such as cancer. Hu et al.[37] examined the expression levels of PCNA and E-cadherin in gastric cancer patients and reported that E-cadherin may play a protective role in the prognosis of patients when both markers are positive. However, tumor proliferation and metastasis increase in PCNA-positive E-cadherin-negative patients.[38] In this study, the PCNA mRNA expression level was minimal in cocultured A549 cells at the 12th h, when the maximum amount of IL-12 was released in the medium. This finding may provide evidence that IL-12-mediated suppression of the cell cycle produces an anticancer response due to a decrease in PCNA levels.

The expression levels of cyclins and CDKs, which are involved in the regulation of the cell cycle, increase in proliferative diseases such as cancer. Gopinathan et al.[17] reported that CDK2 and cyclin A2 knockout in mice reduced tumorigenesis. Dobashi et al.[15] studied lung carcinomas and reported that excess CDK2/cyclin A2 expression in malignant areas was associated with a poor prognosis. Unlike PCNA, the increase in cyclin A2 and CDK2 expression at the 12th h in cocultured A549 lung cancer cells indicated that these cell cycle markers may not be affected by the release of IL-12. However, the decrease in cyclin A2 and CDK2 expression after 24 h of incubation in cocultured A549 cells suggested that IL-12 levels may affect these cell cycle markers after affecting PCNA. Other studies have reported that PCNA is activated from the middle of the G1 phase to the end of the S phase of the cell cycle, whereas cyclin A2 and CDK2 are activated from the S phase to the middle of the G2 phase.[22,39] These cell cycle phases, in which PCNA, cyclin A2, and CDK2 are functional, might explain why PCNA is downregulated earlier than cyclin A2 and CDK2. An increase in PCNA levels following 24 h of coculture might be important for DNA repair in the G2 phase of the cell cycle when DNA replication ceases. Although A549 cells had the highest IL-12 levels at the 24th h compared to all experimental groups, cyclin A2 and CDK2 were not downregulated in A549 cells cultured alone. IL-12 secreted by A549 cells that are cultured alone starts autocrine proliferative signaling, which is widely observed in cancer cells.[40] These findings

suggest the importance of culturing lung cancer cells with MSCs in a coculture since these markers are downregulated in cocultured A549 cells.

CONCLUSION

Systemic administration of IL-12 in patients is toxic, and there are no data related to the spontaneous release of IL-12 in lung cancer cell culture studies. Therefore, we determined the level of IL-12 released from cocultured and cultured MSCs and A549 cells. In this study, novel data showed that the expression of the cell cycle markers PCNA, cyclin A2, and CDK2 decreased in cocultured A549 cells when IL-12 levels were high but not in A549 cell cultures alone; these findings highlighted the importance of coculturing lung cancer A549 cells with MSCs. These results provided insights into the use of MSC-mediated IL-12-related anticancer therapies as alternative methods for administering IL-12.

Ethics

Ethics Committee Approval: Not necessary.

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: M.B.I.Y., S.T., Concept: M.B.I.Y., S.T., Design: M.B.I.Y., S.T., Data Collection or Processing: M.B.I.Y., S.T., Analysis or Interpretation: M.B.I.Y., S.T., Literature Search: M.B.I.Y., S.T., Writing: M.B.I.Y., S.T.

Conflict of Interest: No conflict of interest was declared by the authors.

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ORIGINAL ARTICLE

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Second Line Therapies After Tyrosine Kinase Inhibitory in Metastatic Renal Cell Carcinoma

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ABSTRACT

Objective: Controversy exists over the choice of 2nd-line drugs after the progression of 1st-line tyrosine kinase inhibitors (TKIs) for patients unable to receive 1st-line immunotherapy. We compared the efficacy of 2nd-line treatments after the progression of 1st-line TKI therapy and determined the factors predictive of its efficacy for the treatment of metastatic renal cell carcinoma (RCC).

Material and Methods: Patients were divided into 3 groups according to 2nd-line treatments: axitinib, everolimus, and nivolumab. The progression-free survival (PFS) rates for 2nd-line treatments (PFS2) and overall survival (OS) rates were calculated. Cox regression analyses were conducted to determine the associations between PFS2 and OS rates and other explanatory variables. In addition, PFS2 was compared in patients whose PFS on 1st-line TKI (PFS1) was ≥6 months (mn) with a <6-mn response for each of the 3 groups.

Results: This study included 82 patients who were diagnosed with metastatic RCC. Fourty-one patients received axitinib, 30 patients received everolimus, and 11 patients received nivolumab as a 2nd-line treatment. PFS2 and OS were statistically similar for all 3 groups. Patients who had PFS1 ≥6 months responded significantly to 2nd-line axitinib treatment compared with those with PFS1 <6 months. Multivariate analyses revealed that only PFS1 <6 months was correlated with poor OS.

Conclusion: PFS2 and OS were statistically similar among second-line axitinib, everolimus, and nivolumab treatments. PFS1 <6 mn was correlated with poor PFS2 and OS.

Keywords: Renal cell carcinoma; axitinib; nivolumab; everolimus

INTRODUCTION

Approximately 1/3 of patients with renal cell carcinoma (RCC) present with metastatic disease at their 1st hospital admission.[1] Metastatic RCC has a poor overall survival (OS) rate, with a 5-year OS rate of 12% in the metastatic stage.[1] Despite current treatments for metastatic RCC, the tumors mostly progress, and only 60% of patients can receive 2nd-line treatments.[2]

RCCs are resistant to cytotoxic chemotherapeutic agents.[3] For example, inactivation of the Von Hippel Lindau gene by the deletion of chromosome 3p causes an accumulation of hypoxy inducible factors.[4] This activates angiogenesis due to increased levels of vascular endothelial growth factor (VEGF).[5] In addition, RCCs are considerably hyperinflamed tumors; high levels of proinflammatory cytokines induce an immune response.[6] Multiple kinase inhibitors and immune checkpoint inhibitors are commonly used for treating metastatic RCC because they are characterized by hypervascularization and an increased immune response. The current 1st-line treatment comprises dual immunotherapy (IO+IO) or immunotherapy and multikinase inhibitor (IO+TKI) combinations.[7] However,

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several patients have not yet received 1st-line immunotherapy. Currently, several choices are available after the progression of first-line VEGF/VEGFR axis inhibitors. One option is continuing the inhibition of the VEGF/VEGFR axis with or without an immune checkpoint inhibitor.[8] Another option is to use dual- or monotherapy immune checkpoint inhibitors in patients in whom immunotherapy has not been used. No consensus exists on the best strategy for 2nd-line treatment. A few patients benefit more from tyrosine kinase inhibitors (TKIs), whereas other patients benefit more from immunotherapies. Patients with longer progression-free survival (PFS) rates to 1st-line TKI treatment may respond better to TKIs than to immunotherapy after progression on 1st-line TKIs. Conflicting data exist concerning this hypothesis.[9,10]

We compared the efficacy of 2nd-line treatments after the progression of 1st-line TKI treatments and determined the predictive factors for the efficacy of 2nd-line treatments in patients with metastatic RCC. In addition, we determined whether 2nd-line TKI treatments are more efficacious for longer PFS rates than 1st-line TKI (PFS1) treatments are compared with immunotherapy and mammalian target of rapamycin inhibitors.

MATERIAL AND METHODS

Study Design and Patient Characteristics

The medical records of patients diagnosed with metastatic RCC who had received 2nd-line treatment at the Kayseri City Hospital and Erciyes University Department of Medical Oncology were retrospectively reviewed between January 2007 and July 2024. Patients under the age of 18 years and those with non-metastatic diseases were excluded from the study.

Patients were divided into 3 groups, namely, the axitinib arm, the everolimus arm, and the nivolumab arm, according to 2nd-line treatments. The following patient characteristics were recorded for each study group: age at diagnosis, gender, histological subtype of the tumor, nephrectomy status, time from diagnosis to metastasis, metastatic site, number of metastatic organs, Memorial Sloan-Kettering Cancer Center (MSKCC) risk score, and the PFS rate of 1st-line TKI treatment. The study was approved by Kayseri City Hospital Non-Interventional Clinical Research Ethics Committee (date: March 14, 2024; no: 20).

Statistical Analysis

Frequencies and percentages (descriptive statistics) were used for categorical variables, and medians (minimum-maximum) were used for continuous variables. The PFS rates for 2nd-line treatments and OS rates were calculated using Kaplan-Meier analysis. Cox regression analyses were conducted to determine the associations between the PFS rates of 2nd-line treatments and other explanatory variables. In addition, Cox regression analyses were performed to determine the associations between OS rates and other explanatory variables. The PFS rates of patients who received 2nd-line treatments were compared with those of patients with PFS ≥6 mn to 1st-line TKI with PFS <6 mn, and Kaplan-Meier analyses were performed for each of the 3 groups. PFS was defined as the beginning time of treatment to death or progression of the disease. OS was defined as the time from the diagnosis of metastatic disease to death or the last control time. $p<0.05$ was considered to indicate statistical significance.

The study protocol adhered to the principles of the Declaration of Helsinki at all stages.

RESULTS

Patients and Patient Characteristics

The study included 82 patients who were diagnosed with metastatic RCC and had received 2nd-line treatment after 1st-line TKI treatment. Forty-one (50%) patients received axitinib as a 2nd-line treatment, 30 (37%) patients received everolimus as a 2nd-line treatment, and 11 (13%) patients received nivolumab as a 2nd-line treatment.

All the patient characteristics are summarized in Table 1.

Progression-Free Survival and Overall Survival

The PFS rate after 2nd-line treatment (PFS2) was 7 months (2.82-11.17) for the axitinib arm, 7 months (5.53-8.46) for the everolimus arm, and 8 months (6.73-9.26) for the nivolumab arm. No significant differences were present between these 3 arms (p=0.50). The OS rate was 21 months (10.98-31.01) for the axitinib arm, 35 months (23.96-46.03) for the everolimus arm, and 59 months (not reached) for the nivolumab arm, with no significant differences between these OS rates (p=0.205) (Figure 1).

The PFS2 rate was 2 months (0.974-3.026) in patients with PFS1<6 months and 9 months (0.339-17.661) in those with PFS1 ≥6 months on axitinib treatment (p<0.001). The PFS2 rates were 3 months (0.00-8.544) in patients with PFS1 <6 and 7 months (5.912-8.088) in those with PFS1 ≥6 on everolimus treatment (p=0.108). The PFS rates were 8 months (not reached) in patients with PFS1 <6 months and 19 months (7.560-30.440) in those with PFS1 ≥6 months on nivolumab treatment (p=0.659) (Figure 1).

Univariate analysis revealed that PFS1 <6 months was associated with poor PFS2 rates, with a hazard ratio of 0.373 (0.198-0.702, p=0.002) (Table 2).

TABLE 1: General characteristics.

	Axitinib, n=41 (50%)	Everolimus, n=30 (37%)	Nivolumab, n=11 (13%)
Age	58 (30-77)	59 (24-77)	67 (36-75)
Age <65 Age ≥65	33 (81) 8 (19)	19 (63) 11 (37)	5 (46) 6 (54)
Gender			
Female	13 (32)	5 (17)	2 (18)
Male	28 (68)	25 (83)	9 (82)
Histology			
Clear cell	36 (88)	26 (87)	11
Other	5 (12)	4 (13)	0
Nephrectomy			
No	6 (15)	9 (30)	4 (36)
Yes	35 (85)	21 (70)	7 (64)
Intervention			
No intervention	4 (10)	8 (27)	3 (27)
Nephrectomy	35 (85)	21 (70)	7 (64)
Embolisation	2 (5)	1 (3)	1 (9)
First line treatment			
Sunitinib	30 (73)	19 (63)	9 (82)
Pazopanib	8 (20)	3 (10)	2 (18)
Sorafenib	3 (7)	8 (7)	0
De novo metastatic disease			
No	17 (42)	12 (40)	3 (27)
Yes	24 (58)	18 (60)	8 (73)
MSKCC risk score			
Favorable	7 (17)	4 (13)	2 (18)
Intermediate	21 (51)	22 (74)	7 (64)
Poor	13 (32)	4 (13)	2 (18)
Liver metastasis			
No	29 (71)	24 (80)	9 (82)
Yes	12 (29)	6 (20)	2 (18)
Lung metastasis			
No	6 (15)	7 (23)	2 (18)
Yes	35 (85)	23 (77)	9 (82)
Bone metastasis			
No	28 (68)	24 (80)	7 (64)
Yes	13 (32)	6 (20)	4 (36)
Brain metastasis			
No	39 (95)	24 (80)	9 (82)
Yes	2 (5)	6 (20)	2 (18)
≥6 months 1st-line PFS			
No	7 (17)	8 (27)	3 (27)
Yes	34 (83)	22 (73)	8 (73)

MSKCC: Memorial Sloan-Kettering Cancer Center.

Furthermore, the univariate analysis revealed that a poor MSKCC score was significantly correlated with a poor OS rate, with a hazard ratio of 2.539 (1.180-5.463, p=0.017), and PFS1 <6 months was correlated with a poor OS rate, with a hazard ratio of 0.252 (0.149-0.426, p<0.001). The multivariate analyses revealed that PFS1 <6 months was correlated with a poor OS rate, with a hazard ratio of 0.229 (0.125-0.420, p<0.001) (Table 2).

DISCUSSION

Metastatic RCCs are vascular and immunogenic tumors for which new therapeutic strategies are being continuously developed. Although 1st-line IO+IO or IO+TKI combinations are recommended therapies for metastatic RCC, certain patients are unable to receive 1st-line immunotherapy, making TKIs an appropriate 1st-line treatment option. Which drug should be used as a 2nd-line treatment after the progression of 1st-line TKIs remains unclear. We demonstrated that the PFS rates associated with 3 drugs, namely, nivolumab, everolimus, and axitinib, were statistically similar to those associated with 2nd-line treatments. The OS rate was not significantly different among these 3 groups. In addition, we demonstrated that PFS1 ≥6 months is an independent prognostic factor for PFS2 and OS. Patients who received 2nd-line axitinib and whose PFS1 was ≥6 months had significantly greater PFS2 rates than patients whose PFS1 was <6 months. No significant differences in PFS2 were noted between patients with PFS1

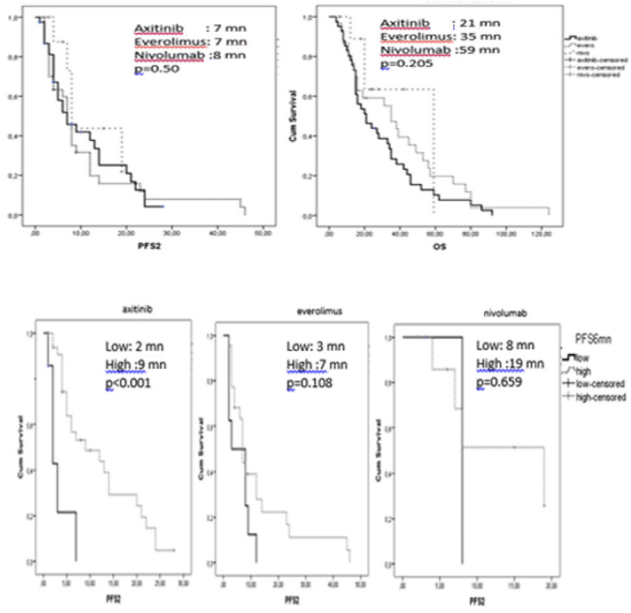

FIGURE 1: PFS2 and OS for axitinib, everolimus and nivolumab arm and progression free survival of second line treatments according to PFS1.

PFS2: Progression free survival-2; OS: Overall Survival

TABLE 2: Univariate and multivariate analyses for PFS2 and overal survival.

Characteristics	PFS2		OS			
	Univariate		Univariate		Multivariate	
	HR, 95% CI	p value	HR, 95% CI	p value	HR, 95% CI	p value
Age	0.995 (0.976-1.015)	0.631	1.004 (0.985-1.024)	0.682		
Gender Female or male	1.017 (0.561-1.845)	0.955	0.791 (0.452-1.384)	0.411		
Nephrectomy No or yes	0.666 (0.382-1.160)	0.151	0.792 (0.456-1.377)	0.408		
De novo metastatic Yes or no	0.839 (0.505-1.394)	0.499	1.381 (0.851-2.241)	0.191		
MSKCC risk score Favorable or intermediate Intermediate or poor	1.017 (0.532-1.942) 0.966 (0.393-2.374)	0.960 0.939	1.20 (0.614-2.343) 2.539 (1.180-5.463)	0.594 **0.017**	1.047 (0.532-2.060) 1.999 (0.913-4.378)	0.894 0.083
Liver metastasis No or yes	1.148 (0.660-1.997)	0.626	0.742 (0.427-1.288)	0.289		
Lung metastasis No or yes	1.330 (0.689-2.565)	0.395	1.711 (0.838-3.493)	0.141		
Bone metastasis No or yes	1.147 (0.638-2.062)	0.646	0.690 (0.405-1.176)	0.173		
Brain metastasis No or yes	1.171 (0.521-2.631)	0.703	1.040 (0.471-2.294)	0.923		
≥6 months 1st-line PFS No or yes	0.373 (0.198-0.702)	**0.002**	0.210 (0.116-0.379)	**<0.001**	0.229 (0.125-0.420)	**<0.001**

PFS2: Progression free survival-2; OS: Overall survival; HR: Hazard ratio; CI: Confidence interval; MSKCC: Memorial Sloan-Kettering Cancer Center.

≥6 months and those with PFS1 <6 months who received 2nd-line everolimus or nivolumab treatments.

Motzer et al.[9] conducted a phase 3 study that included 821 patients with advanced RCC and compared nivolumab and everolimus treatments after the progression of 1st- or 2nd-line antiangiogenic therapy. They reported that the median PFS rates were 4.6 months and 4.4 months, respectively, with nivolumab and everolimus treatments (p=0.11). This finding is consistent with that of our study. Our PFS rates were higher than those reported by Motzer et al.[9] for both nivolumab and everolimus treatments. The median OS rates were 25.0 months and 19.6 months in the nivolumab and everolimus groups, respectively, and these values were significantly different. In our study, the OS rates were 59 months and 35 months for the nivolumab and everolimus treatments, respectively. Although a 24-month OS rate difference existed between the everolimus and nivolumab treatment groups, this difference was not significant. The 1st reason for this result could be the small size of our study. Second, crossover was present in our study. Another difference from the other study was that certain patients had received 2 lines of antiangiogenic agents before their treatment. In our study, all patients received only 1 line of antiangiogenic agent. Another study revealed prolonged survival with nivolumab treatment compared with everolimus treatment, irrespective of the MSKCC score. Our univariate analysis revealed that the MSKCC score was an independent prognostic marker. However, this result was significant in multivariate analyses. Both uni- and multivariate analyses revealed longer PFS1 as the only independent prognostic factor for PFS and OS. The CheckMate 025 trial demonstrated improved PFS rates in patients who received nivolumab treatment compared with those who received everolimus treatment.[11] Pehlivan et al.[12] compared second-line axitinib and nivolumab treatments. They reported higher PFS and OS rates with second-line nivolumab treatment than with axitinib treatment. In our study, more patients had poor MSKCC scores in the nivolumab arm group than in both the axitinib and everolimus arm groups. Instudy from Pehlivan et al.[12] poor MSKCC scores were similarly found. Although a high rate of poor MSKCC scores in the nivolumab arm group was noted in our study, the OS rate was higher in the nivolumab arm group. However, the results were not significantly different. The nivolumab arm group did not report sufficient progression or death; therefore, the PFS and OS results were immature.

Busch et al.[13] conducted a study comparing 2nd-line everolimus and TKI treatments and reported no statistically significant difference in PFS between the 2 groups. However, sunitinib or sorafenib was used as a 2nd-line TKI in their study. In contrast, the present study exclusively utilized axitinib as a 2nd-line TKI following prior TKI failure.

These findings indicate that PFS1 serves as an independent prognostic marker for both PFS2 and OS. Additionally, patients with PFS1 ≥6 months demonstrated a statistically significant response to 2nd-line axitinib treatment compared with those with PFS1 <6 months. However, this statistical significance was not observed in the everolimus and nivolumab treatment arms. A subanalysis of the phase III Axis trial revealed that patients with prolonged responses to 1st-line cytokine therapy exhibited improved survival outcomes with 2nd-line axitinib treatment.[10,14] However, a prolonged response to 1st-line sunitinib did not influence the response to 2nd-line axitinib. In that study, responders were defined as those who achieved a complete or partial response. In the present study, patient groups were categorized on the basis of PFS1 ≥6 months or PFS1 <6 months following 1st-line treatment. Among patients receiving prior sunitinib, the median duration of 1st-line therapy in the axitinib group was 9.7 months, which was used as the cut-off for a prolonged response. Similarly, Seidel et al.[15] identified 1st-line PFS duration as an independent prognostic marker, with a cut-off of 6 months, which aligns with the findings of the present study.

To the best of our knowledge, this study is the only 1 comparing 3 distinct second-line agents with different mechanisms of action-nivolumab, axitinib, and everolimus. However, several limitations must be acknowledged, including the retrospective design and the relatively small study population.

CONCLUSION

No statistically significant differences were observed in PFS or OS among 2nd-line treatments with axitinib, everolimus, or nivolumab. Axitinib treatment significantly improved PFS2 in patients with PFS1 ≥6 months compared with those with PFS1 <6 months. However, in the nivolumab and everolimus groups, PFS2 rates did not significantly differ on the basis of PFS1 duration. This finding should be interpreted with caution due to the limited sample size.

Ethics

Ethics Committee Approval: The study was approved by Kayseri City Hospital Non-Interventional Clinical Research Ethics Committee (date: March 14, 2024; no: 20).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.D., A.K.D., Ş.Y.D., O.B., M.İ., M.Ö., Concept: E.D., M.Ö., Design: E.D., S.T.F., A.K.D., Ş.Y.D., O.B., M.İ., M.Ö., Data Collection or Processing: E.D., Ş.Y.D., A.K.D., S.T.F., Analysis or Interpretation: E.D., Literature Search: E.D., S.T.F., A.K.D., Ş.Y.D., Writing: E.D., Ş.Y.D.

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ORIGINAL ARTICLE

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Evaluation of 2024 Turkish Medical Oncology Board Exam with ChatGPT

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ABSTRACT

Objective: This study aims to assess ChatGPT-4's performance on the Turkish Medical Oncology Board Exam questions, highlighting its potential uses and limitations in medical specialty evaluations.

Material and Methods: ChatGPT-4 was presented with each question from the 2024 Turkish Medical Oncology Proficiency Exam. Answers were determined to be correct or incorrect by comparison with the official answer key.

Results: The overall accuracy of ChatGPT-4.0 in this study was 64% out of 100 questions. For the fact-based questions (45 items), which require knowledge of specific information, such as molecules and side effects, ChatGPT-4o demonstrated an accuracy of 75.5%, with 34 correct responses. However, in the case-based questions (55 items) that require clinical judgment, its accuracy dropped to 54.5% (correct responses of 30). All these results highlight strengths of ChatGPT-4o on fact-driven questions but expose its limitations in scenarios needing nuanced decision-making.

Conclusion: Oncological clinical decision-making necessitates a nuanced approach that extends beyond standardized guidelines, integrating individual patient variables such as medical history, comorbidities, and therapeutic responses. While artificial intelligence (AI) systems demonstrate proficiency in processing guideline-driven data, they exhibit limitations in contextual clinical judgment requiring physician expertise. This study observed ChatGPT-4's superior performance on knowledge-based assessments (75.5% accuracy), attributable to its training on the American Society of Clinical Oncology/the European Society for Medical Oncology frameworks. However, its accuracy declined significantly in case-based evaluations (54.5%), highlighting challenges in personalized care integration. These findings underscore the indispensable role of clinician judgment in navigating complex, individualized treatment landscapes. Enhancing AI's clinical utility requires training on real-world patient data, though ethical constraints-particularly General Data Protection Regulation compliance-limit access to such datasets. Institution-specific AI tools leveraging anonymized records may bridge this gap, pending technological and regulatory advancements.

Keywords: Medical oncology; medical board exams; large language model; clinical reasoning

INTRODUCTION

Recent advancement in NLP, especially the introduction of ChatGPT-4 and subsequent models, has vastly changed medical education and assessment by increasing the capability to address complex board examination questions.[1,2] ChatGPT-4o, updated to include guidelines from major professional bodies such as the American Society of Clinical Oncology and the European Society for Medical Oncology, demonstrated improved clinical reasoning skills and positioned itself as a promising support tool for practicing clinicians and trainees alike.[3,4] ChatGPT-4's ability to pass high-stakes examinations, as demonstrated in the report by Kung et al.[4] on the successful performance of ChatGPT-4 in the United States Medical Licensing Examination, further points to its potential value in medical settings.[5] While earlier versions were particularly good at fact-based questions and could not perform well in case-based scenarios that required subtlety in judgment, the recent improvements have enhanced the capability of ChatGPT in understanding context.[6,7] These newer versions also have their limitations with regard to distinguishing subtle clinical cues, hence a need for continued research to refine their use in medical training.[3] In this context, the

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current study has aimed at the performance of ChatGPT-4o at addressing questions from the Turkish Medical Oncology Board Exam, reflecting both the potential benefits and challenges encountered in specialty assessments.

MATERIAL AND METHODS

In this study, ChatGPT-4 was systematically tested using the 2024 Turkish Medical Oncology Board Examination questions. The examination questions were received from the official website of the Turkish Society of Medical Oncology (www.kanser.org) and were presented to ChatGPT-4o without translation and verbatim, to ensure their original context and integrity were preserved. A total of 100 questions were analyzed, consisting of 55 case-based questions (clinical scenarios requiring decision-making) and 45 knowledge-based questions (factual recall of drug mechanisms, side effects, and guideline recommendations).

Each question was entered into ChatGPT-4 line by line and transferred to the ChatGPT-4 without any adaptation or translation as it appeared on the Turkish Society of Medical Oncology platform (www.kanser.org). The responses of the model were noted and then compared with the official answer key published by the Society. Responses were classified as correct or incorrect based on this comparison, thus allowing a direct evaluation of ChatGPT-4's accuracy across question types.

Case-based questions evaluated the model's capability to synthesize clinical facts and suggest patient-specific management strategies, while knowledge-based questions included factoid recall, such as medication mechanisms, or guideline-endorsed protocols. These items were analyzed for accuracy rates of the two categories to find the difference in performance.

This study aims to determine the degree to which ChatGPT-4 can simulate clinical reasoning in oncology and to outline the usefulness and limitations of its application in the assessment of medical oncology competence.

RESULTS

Analysis of ChatGPT-4o's response to the 2024 Turkish Medical Oncology Board Exam demonstrated different performances between clinical and direct knowledge-based questions. Out of 100 questions, 55 were scenario-based clinical questions, while 45 were direct information-focused ones. ChatGPT-4o answered 64% of all questions correctly; looking at it from another point of view, there was an obvious difference between the types of questions. On the direct knowledge questions, which required recalling specific facts such as drug mechanisms or side effects, ChatGPT-4o performed well, with 34 correct answers out of 45 for a 75.5% success rate within the knowledge category (Figure 1).

There is evidence of the model's strength in retaining and recalling guideline-based medical information.

In contrast, ChatGPT-4o's performance on clinical questions, which demand a more interpretative, case-based approach, demonstrated reduced accuracy. The model correctly answered 30 of 55 clinical questions for a success rate of 54.5%. The lower accuracy observed is consistent with what has been seen in artificial intelligence (AI)-driven models whenever there are complex, individualized treatments where human clinical judgment and contextual understanding come into play. These results emphasize that, while the AI has shown proficiency in direct knowledge recall, there are still many challenges for it to overcome in effectively adapting guideline-based information to nuanced clinical contexts.

DISCUSSION

The objective of this study is to evaluate the performance of ChatGPT-4 on the 2024 Turkish Medical Oncology Proficiency Examination, both in terms of recall of facts and clinical judgment. The performance of the model was considerably better on fact-type questions (accuracy 75.5%) while overall accuracy was 64%-particularly for questions related to oncological drugs and side effects. The results were consistent with earlier studies by Barbour and Barbour[6] and Kung et al.[4] showing that artificial intelligence models perform better on knowledge-based, structured questions. Their performance reduced to 54.5% when case-based questions were present, which required critical thinking as well as patient-specific decision-making.

One of the major reasons for this divergence is the complexity of individualized patient management. While the answers produced by ChatGPT-4 are based on documented oncology literature and guidelines, such as those released by the NCCN,

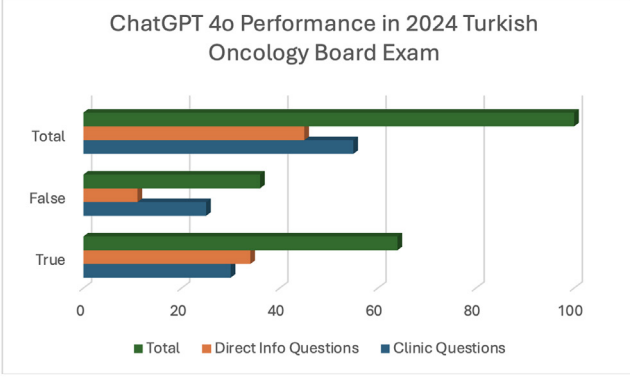

FIGURE 1: ChatGPT performance in 2024 Turkish Oncology Board Exam.

the American Society of Clinical Oncology (ASCO), and the European Society for Medical Oncology (ESMO), real-time medical decision-making goes beyond predetermined protocols. Physicians need to consider a number of variables, including the patient's comorbidities, functional status, socioeconomic status, access to healthcare services, and health insurance plans because each of these variables affects treatment decisions but is not directly addressed by clinical guidelines. Thus, although these guidelines are useful sources, they cannot substitute for physicians' clinical judgment, particularly in complex cases.

These issues have also been found in other branches of medicine. A study in urology[7] shows how AI is not able to deliver standardized responses to intricate, patient-specific situations. In addition, a study concluded that AI was not flexible in clinical decision-making, substantiating its more structured nature.

The findings of the study indicate that, while ChatGPT-4.0 performed well in evidence-based assessments, it faced difficulties with case-based reasoning. According to a study,[7-10] there is still more to be done to enhance the ability of artificial intelligence for balancing theoretical knowledge and the dynamics of real-world clinical situations, particularly in oncology.

CONCLUSION

Clinical decision-making in oncology is not simply following guidelines-it is weighing each patient's unique medical history, comorbidities, and response to treatment. Two patients may share the same cancer type and stage but could need different therapeutic approaches depending on age, genetic factors, or overall health status. AI models like ChatGPT-4o excel at reading medical literature and standard operating procedures, but are behind when faced with complicated, case-by-case decisions that only a physician's experience can supply.

ChatGPT-4 demonstrated remarkable efficacy in answering knowledge-based questions on the Turkish Medical Oncology Proficiency Exam, owing mainly to its reliance on the guidelines provided by ASCO and ESMO.

While ChatGPT-4.0 was good at knowledge-based questions, it struggled with case-based situations involving clinical judgment and individualized patient care. Even with further advancement of artificial intelligence, the complexity of oncology decision-making continues to heavily rely on the experience of doctors to assess individual patient variables and decide on the best treatment regimens.

To perform better in this area, the AI would need to rely on actual patient cases rather than relying solely on medical guidelines and textbooks. This does create ethical and legal problems, particularly with patient privacy laws such as General Data Protection Regulation, limiting access to actual clinical data. Due to such restrictions, general AI models such as ChatGPT-4 could always fall short in patient-specific decision-making.

A better option would be to develop AI models in hospitals or medical institutions, where anonymized patient information could be used subject to privacy legislation. With enhanced technology and declining costs of computing, these expert models might give more accurate clinical guidance without breaching patient confidentiality.

Ethics

Ethics Committee Approval: Ethics committee approval is not required for this study.

Informed Consent: Informed consent approval is not required for this study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.G.K., O.Ü.Ü., Concept: E.G.K., O.Ü.Ü., Design: E.G.K., O.Ü.Ü., Data Collection or Processing: E.G.K., O.Ü.Ü., Analysis or Interpretation: E.G.K., O.Ü.Ü., Literature Search: E.G.K., O.Ü.Ü., Writing: E.G.K., O.Ü.Ü.

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REVIEW

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Application of Synthetic Antigen-Encoded *Escherichia coli* Nissle 1917 Probiotic-Guided PD-1/CD28 Receptor-Integrated CAR-T Cell Therapy as Targeted Therapy for Colorectal Cancer: A Literature Review

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ABSTRACT

Colorectal cancer is a leading cause of cancer-related deaths. The traditional therapeutic strategies, such as chemotherapy and surgery, which are generally used to treat colorectal cancer are invasive and often accompanied by significant side effects. Chimeric antigen receptor-T (CAR-T) cell therapy is effective, particularly in treating hematological malignancies; however, the treatment of solid tumors such as colorectal cancer is difficult. These challenges include poor targeting, loss of function, inadequate expansion, and short-lived persistence of CAR-T-cells. Some researchers have developed a novel approach to overcome these limitations by using the probiotic bacterium *Escherichia coli* Nissle 1917 (EcN) and integrating PD-1/CD28 receptors into CAR-T-cells. EcN bacteria naturally target the tumor microenvironment and can be genetically engineered to release synthetic antigens at the tumor site. This improves the targeting ability of CAR-T-cells, ensuring that they localize and activate precisely where needed. Additionally, PD-1/CD28 receptor integration enhances the efficacy of CAR-T-cells by converting inhibitory signals into costimulatory signals, thus increasing the activation, proliferation, and persistence of T-cells. This approach has shown promising preclinical results, indicating improved targeting, activation, and longevity of CAR-T-cells in solid tumors. Researchers should next focus on optimizing bacterial engineering, enhancing CAR-T-cell design, and conducting rigorous clinical trials to validate the safety and effectiveness of this combined therapy. Their findings may revolutionize treatment for colorectal cancer.

Keywords: Synthetic antigen; *E. coli* Nissle 1917; colorectal cancer; PD-1/CD28; CAR-T-cells

INTRODUCTION

Cancer has a high death rate worldwide. Colorectal cancer is the second deadliest cancer in the world. In 2018, about 1.8 million new cases of colorectal cancer were expected, with the death toll reaching about 881,000.[1] The development of colorectal cancer can be triggered by genome instability, that results in genetic and epigenetic mutations, which can transform normal glandular epithelial cells into benign tumors (neoplasms) and can further transform into an invasive type of cancer (carcinoma).[2]

The available treatment options include surgery, chemotherapy, and radiotherapy; however, these techniques are invasive and have significant side effects and resistance. Therefore, immunotherapy is a promising alternative.[3,4] The complexity of cancer treatment, as well as the intricate interactions between immune cells and cancer cells, highlights the need for targeted immunotherapy, such as chimeric antigen receptor T-cell (CAR-T) therapy. Unlike the typical mechanism of action of T-cells, CAR-T-cells are engineered to identify targets without relying on the expression of the major histocompatibility complex (MHC). CAR-T-cells can express

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CARs that target specific antigens present on the surface of tumor cells.[4] CAR-T-cell therapy was found to effectively treat blood-related cancers, including multiple myeloma, non-Hodgkin lymphoma, and acute lymphoblastic leukemia.[3,4] Although CAR-T-cell therapy can treat hematological malignancies, challenges in the context of solid tumors remain. These challenges include the expression patterns of tumor-associated antigens (TAAs) in solid tumors and the phenomenon of T-cell exhaustion.[4-6] This highlights that new methods are needed for CAR-T-cell therapy. Such methods should not depend on tumor antigens and should increase the activity of active CAR-T-cells, including CAR-T-cell therapy, integrated immunostimulatory fusion protein (IFP) PD-1/CD28, and guided synthetic antigen-encoded probiotics.[4,6]

The probiotic *Escherichia coli* Nissle 1917 (EcN) is based on the ability of bacteria to colonize tumor cells. It is specifically localized in the nuclei of hypoxic tumor cells and is safe for human use.[7,8] These probiotic bacteria are engineered to produce and release synthetic antigens, which are subsequently responded to by CAR-T-cells.[6]

The use of IFP is a new strategy to overcome T-cell exhaustion, which is triggered by the interaction between programmed death-ligand 1 (PD-L1) and the programmed cell death protein 1 (PD-1) receptor, which reduces the function and activity of T-cells.[5] IFP consists of the extracellular domain PD-1 protein, as well as the transmembrane and intracellular domains of the CD28 protein.[5,9] Using this strategy, inhibitory signals can be converted into stimulatory signals, and thus it is effective in overcoming CAR-T-cell immunosuppression.[5] This literature review comprehensively examines the refinement of PD-1/CD28 receptor-integrated CAR-T-cell therapy guided by synthetic antigen-encoded *EcN* 1917 probiotics as a new modality for treating colorectal cancer.

MATERIAL AND METHODS

This article was written after conducting a comprehensive and selective literature review. The relationships between each library were searched through online scientific database search engines such as PubMed, ScienceDirect, Google Scholar, and the Publish or Perish application by applying the Boolean "AND" and "OR" logic. The keywords used in this search included CAR-T-cells, PD-1/CD28, *EcN* 1917, colorectal cancer, and synthetic antigens. The inclusion criteria set were references with a publication period of the last 10 years (2014-2024). This literature review also had certain exclusion criteria. We did not include studies published before 2014 to maintain data relevance.

Pathophysiology of Colorectal Cancer

There are three main molecular pathways related to colorectal cancer tumorigenesis, namely chromosome instability, mismatch repair, and CpG hypermethylation pathways. Imbalance between oncogenes and tumor suppressor genes leads to chromosomal instability, such as mutations that occur in adenomatous polyposis coli (APC).[10] Mutations in the APC gene lead to the translocation of beta-catenin into the cell nucleus, which then activates Wnt signaling. Translocation in the nucleus results in heterodimerization with transcription factors, which promotes intestinal epithelial cell proliferation and tumorigenesis. Moreover, Wnt signaling plays a role in activating several genes related to tumorigenesis. After APC mutation, KRAS is activated, which influences the activation of Raf-MEK-ERK, phosphoinositide 3 kinase (PI3K), and NF-kB, ultimately promoting cell proliferation. In the final stages of tumorigenesis, mutations occur in the TP53 gene, thus promoting tumor development.[11]

In contrast to the chromosome instability pathway, the mismatch repair pathway is characterized by hypermutation in somatic DNA, including mutations involved in DNA mismatch repair, such as epithelial cell adhesion molecule (EpCAM), resulting in an accumulation of repeated gene mutations that lead to microsatellite instability (MSI).[10,11] Tumor cells with the MSI phenotype are unable to recognize and repair mismatched DNA. The cell maintains and replicates the mutation. MSI mutations include mutations in the TGFB receptor-2 (TGFBR2) gene, which encodes a protein that inhibits intestinal epithelial cell proliferation. MSI mutations also occur in other genes that encode proteins that regulate cell proliferation, apoptosis, and DNA repair.[11]

Tumors can also develop through hypermethylation of CpG islands, which are a collection of cytosine/guanine bases connected by phosphodiester bonds and are often found in the promoter regions of genes. Hypermethylation of CpG islands in the promoters of tumor suppressor genes triggers tumor development. An example of a gene that undergoes promoter hypermethylation is MLH1, which functions in DNA repair (Figure 1).[11]

CAR-T-Cell Therapy

CAR-T cell therapy is a renewable type of immunotherapy for treating non-solid and solid cancers. This treatment technique utilizes the extraction of normal T-lymphocytes from the patient's body through leukopheresis.[12] Next, a specific receptor (CAR) is integrated into T-cells to increase the potential of immunotherapy. Modified T-cells are multiplied using *in vitro* media. Then, the CAR-T-cells are administered back to the patient to attack and work on the cancer location specifically based on the CAR target.[12,13]

CAR-T cell therapy is widely applied in cases of hematological malignancies such as leukemia and lymphoma. Research by Ali et al.[14] showed a complete remission on 82% of the

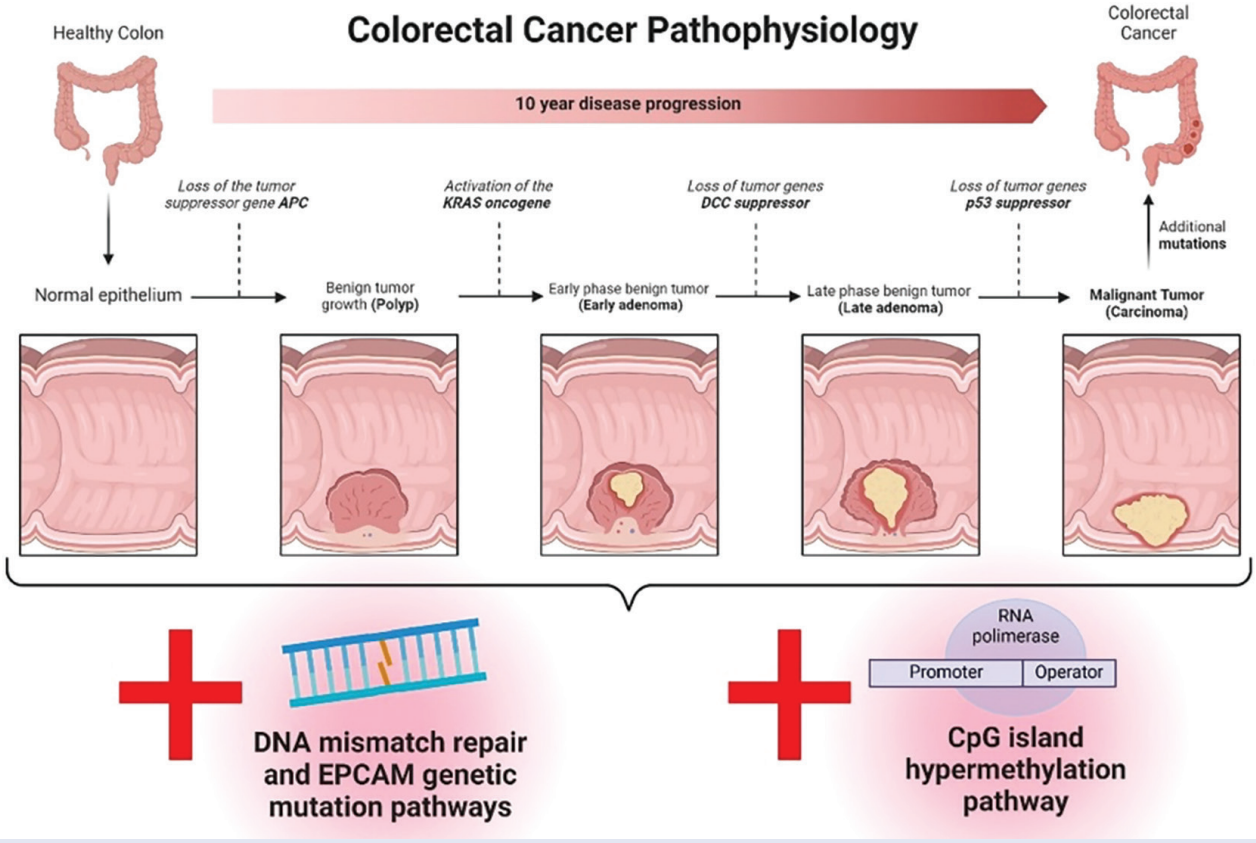

FIGURE 1. Pathophysiology of colorectal cancer.[11]

patients suffering from leukemia and lymphoma. Moreover, no excessive side effects were found due to cytokine release syndrome (CRS). CAR-T cell therapy is also used to treat solid cancer cases based on specific antigen markers that match the characteristics of the type of cancer. The markers that are generally used include CD133 and carcinoembryonic antigen (CEA).[15] The CEA and CD133 proteins are predominantly found in the colon. These two proteins are candidate molecular targets in the treatment of colorectal cancer via CAR-T-cells.[16] The combination of CEA with an antibody against the CD30 marker is the most effective strategy for treating colorectal cancer. In clinical trials, administering CEA CAR-T-cells caused seven out of 10 patients with metastatic colorectal cancer to achieve stable conditions within 30 weeks.[17] The ability of the CD133 component to eradicate cancer stem cells (CSCs) is also supported by the finding that 14 of 21 hepatocellular carcinoma patients achieved stable conditions for nine months on average.[18]

CAR-T cell immunotherapy is extremely suitable for treating patients with non-solid cancer. This is due to several reasons related to the weakness of CAR-T-cells in solid cancer treatment. CAR-T-cells cannot reach their maximum potential in the solid tumor microenvironment (TME) because T-cells must have strong extracellular matrix degradation capabilities to reach the site of the tumor. Solid cancer is also known for its constituent components, which are layers of extracellular matrix with tumor-associated fibroblast, collagen, and proteoglycan components, which make it very difficult for T-cells to penetrate.[19] The tumor environment also strongly influences T-cell activity, especially in the presence of cytokines, soluble proteins, and various ligand components, such as CTLA-4 and PD-L1, which can reduce the activity of T-cells. These components activate anergic and apoptotic conditions in CAR-T-cells, which prevents them from performing their functions.[20] Modification of CAR components specific to TAAs is also difficult because TAAs in solid cancer can cause cross-reactions and non-target specific actions with normal cells. This results in high rates of toxicity and product failure following CAR-T-cell therapy (Figure 2).[21-23]

The Potential of EcN 1917 as a Carrier of Synthetic Antigen for CAR-T-cells in Colorectal Cancer

EcN is a serum-sensitive probiotic that does not produce enterotoxins nor cytotoxins which has been approved for use in the treatment of diarrhea and ulcerative colitis. Two types of plasmids, pMUT1 and pMUT2, from EcN are considered

stable and can be used in genetic recombination.[7] This allow EcN to be genetically modified to deliver drugs to disease sites. Thus, such advantage can be utilized to increase the targeting ability of immune cells in targeted therapy for malignant diseases such as cancer.[7,24]

A study investigated the effect of EcN, under the brand name Mutaflor, administered using oral route on mice with colorectal adenomas. It revealed a more localized population of EcN in the tumor area through lipopolysaccharide staining of EcN bacteria.[25] Moreover, experiments on colorectal cancer model mice were also conducted to test the effectiveness of integrated EcN delivery of the cytokine GM-CSF and the nanoantibodies PD-L1 and CTLA-4. Histological examination revealed a 47% decrease in the size and number of tumor cells. This finding highlighted the potential of EcN in the colonization and degradation of colorectal cancer.[26,27] This ability of EcN can be used to deliver synthetic CAR-T antigens in targeted therapy for colorectal cancer.[6]

Genetic modifications applied to EcN for guiding CAR-T-cell involve various types of synthetic antigen components. One experimental result revealed that synthetic antigen consisting of heparin binding domain originating from placental growth factor-2 (PlGF-2) and connected with superfolder green fluorescent protein (sfGFP) via a glycine-serine linker has a great potential. The reason lies in the fact that the specific diffusion of the synthetic antigen in the TME with its unique fibronectin and collagen population will limit the CAR-T-cells to only act inside the TME, thus increasing the safety.[28,29] In addition, using the aforementioned synthetic antigen revealed a more effective activation period extension of the CAR-T-cells.[30,31] As a response to these components, CAR modification involving several appropriate antibodies is also needed for T-cells. Specific antibodies against sfGFP in CARs can be used and linked with CD28 and CD3 domains by IgG4 linker.[31]

The mechanism of EcN and CAR-T-cells in colonization and immunological activation cannot be separated from the mechanism of controlled bacterial replication and multiplication. When EcN with specific antigens is administered and colonizes the tumor site, EcN grows to a certain bacterial population density threshold (quorum threshold). In detecting the quorum threshold, several sensing genes, such as luxI and ϕX174E, play a role. Next, some of the bacteria in the population undergo lysis and release specific antigens, which are targeted by CAR-T-cells. The population that is not lysed continues to grow until it reaches the quorum threshold and repeats the cycle. This mechanism is known as the synchronized lysis circuit (SLIC).[7,32] Studies on the colonization ability of EcN can be found in Table 1.

To determine the targeting ability of CAR-T-cells, Vincent et al.[33] constructed dye-linked sfGFP-PlGF-targeting CAR-T-cells. Then, the CAR-T-cells were incubated with the MDA-MB-468

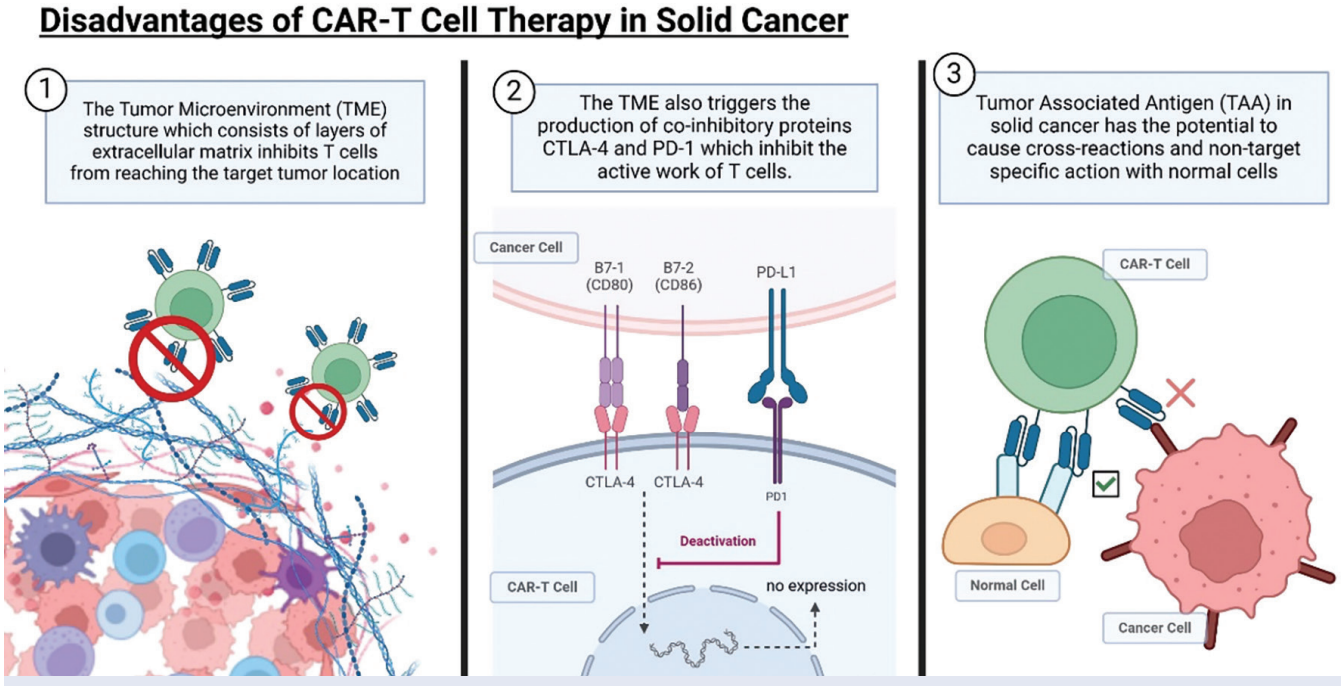

FIGURE 2. Disadvantages of CAR-T cell therapy in solid cancer.[19-23]

CAR-T: Chimeric antigen receptor T.

cell line that was treated with pure sfGFP-PlGF synthetic antigen (100 ng/mL) and observed. The results were then compared to those of the control sample.

The findings showed that cluster formation was more prominent in the group treated with pure synthetic antigen than in the diGFP group. The large number of clusters indicated the number of sfGFP-PlGF antigen receptors that formed synapses on target cells after the pure synthetic antigen was administered. Synapse formation was lower in the diGFP group than in the pure synthetic antigen group because the diGFP group did not have the PlGF domain, which functions to attach synthetic antigens to tumor cells.[33]

Cytotoxicity assessment of sfGFP-PlGF-targeting CAR-T-cells was performed using the luciferase lysis assay. Cytotoxicity assessment was conducted on several cancer cell lines with an effector-to-target (E:T) ratio of 3:1 and incubated with a synthetic antigen (100 ng/mL) for 20 h. The results showed that the level of cytotoxicity (in RLU) in all cell lines treated with the pure synthetic antigen was highly significant compared to that in the control cells treated with PBS ($p<0.0001$). These findings indicated that synthetic antigens act as universal antigens for sfGFP-PlGF-targeting CAR-T-cells regardless of the tumor type and origin.[33]

Assessment of the antitumor activity of sfGFP-PlGF-targeting CAR-T-cells *in vivo* was performed using NSG mice with a Nalm6 cell line xenograft model. Groups of mice then received intratumor injections of EcN (1×10^5 CFU). Intratumor administration of sfGFP-PlGF-targeting CAR-T-cells (2.5×10^6 CFU) was performed two days after EcN was injected, while the control group was administered PBS.[33] The results on day 28 after treatment revealed a decrease in tumor growth in the sfGFP-PlGF-encoded EcN group which tumor volume was <600 mm^3. This result was significantly different from that of the normal EcN group which tumor volume was >600 mm^3 ($p<0.01$). The results in the sfGFP-PlGF group were also highly significantly different from those in the diGFP-encoded EcN group and the control group which tumor volume was >1,200 mm^3 ($p<0.0001$). This significant difference between the diGFP and sfGFP-PlGF groups matched the results of the cytotoxicity test, which further validated the role of the PlGF domain in attaching synthetic antigens to tumor cells. Additionally, sfGFP and diGFP levels in serum were estimated by ELISA on day 14 after EcN injection, and the results revealed a significant difference between the serum levels of sfGFP (<0.02 ng/mL) and diGFP (>0.06 ng/mL) ($p<0.01$). These findings indicated that sfGFP-PlGF can reduce the systemic release of the synthetic antigens. The interferon (IFN)-γ and TNF-α levels also increased significantly ($p<0.01$) in the sfGFP-PlGF group compared to their levels in the control group. These findings further supported the increase in antitumor activity *in vivo*.[33]

TABLE 1: Studies of EcN colonization ability in tumor tissue.

EcN variation	Subject	Method (route, dosage, period)	Results
EcN is normal	Eight female NMRI nude mice with head and neck squamous cell carcinoma model	Intravenous, 1×107 CFU, 3 days	The ratio of bacterial colonization in tumor tissue and normal tissue is almost 1000:1.[47]
EcN luxCDABE cassette encoder (EcN-lux)	ApcMin/+ mice as a precursor model for colorectal cancer	Oral, 1010-1011 CFU/mL, 7 weeks	Bioluminescent bacteria are more abundantly observed in the distal colon where the adenoma burden is greatest.[26]
EcN is normal	BALB/c mice bear 4T1 tumors	Intravenous, 2×104 CFU, 3 days	2 out of 4 mice had tumors colonized with bacteria.[48]
EcN with pMUT-gfp Knr modification	Five BALB/c mice with 4T1 breast cancer cell line xenograft with/without pretreatment with antibiotics	Intranasal, 1×107 CFU, once every 3 days for 18 days	The results of a study of tumor samples showed that there were ~50 times more EcN colonies in the group pretreated with antibiotics, with the highest being 7.37×103±3.39×102 CFU per 1 g of tumor tissue.[49]
Normal EcN and EcN conjugated CA-Dox-Hyd-SH/AuNRs	BALB/c nude mice with MCF-7 breast cancer cell line xenograft	Intravenous, 2×108 CFU, Injection only on the first day	In the group injected with EcN, there was a high accumulation of EcN in tumor tissue on days 2 to 4 after EcN injection. High accumulation of EcN also occurs in the kidneys and liver.[50]
EcN with modified pMut1 plasmid	CB6F1 mice with CT26 colorectal cancer cell line xenograft	Intravenous, 2×108 CFU, injection only on the first day	In the group given modified EcN, there was a significant increase in IL-2 cytokines in tumor tissue compared to the normal EcN group ($p\leq0.001$).[51]

EcN: *E. coli* Nissle 1917.

The adaptive response of the endogenous immune system was assessed using three groups of immunocompetent mouse models of MC38 colorectal cancer cell line xenografts at two different sites. Then, one of the tumors from each group was intratumorally-injected with PBS, normal EcN, and sfGFP-PlGF-encoded EcN (2×10^6 CFU). Then, all groups were intratumorally injected with 1.5×10^6 CFU of sfGFP-PlGF-targeting mouse CAR-T-cells on day 2 and 5 after EcN was administered.[33] The results on day 23 after the administration of EcN sfGFP-PlGF at one tumor site alone revealed not only the inhibition of tumor growth at the injection site but also a reduction in tumor growth at the other and more distal site (>200 mm^3; $p<0.01$) compared to those of the control group and the normal EcN group (~600 mm^3). The results of the immunophenotyping of tumor samples at the site injected with EcN sfGFP-PlGF revealed a significant increase in CD69 expression on CD8+ cells and conventional T-cells (CD4+Foxp3-) compared to that in the control group. Significant differences were also recorded in the increased frequencies of Ki67+ ($p<0.05$) and CD44+ ($p<0.01$) conventional T-cells. Based on these results, Vincent et al.[33] confirmed that administering sfGFP-PlGF-encoding EcN and sfGFP-PlGF-targeting CAR-T-cells can propagate endogenous immune cells, enabling them to induce a systemic antitumor response.

Programmed Cell Death-1 (PD-1) Inhibitory Agents on T Lymphocyte Cell Immune Activity

Under normal conditions, CD8+ cytotoxic T-cells and CD4+ helper T-cells have antigen receptor components that are associated with costimulatory and coinhibitory molecules. Activating these components results in the activation or tolerance of T-cells.[34] The most common coinhibitory secondary signaling component found on T-cells is PD-1, also known as CD279, and cytotoxic T lymphocyte antigen 4 (CTLA-4). When T-cells are activated, PD-1 is expressed on the cell surface and interacts with the PD-L1 ligand (CD274) found on target cells or tumors. The interaction of PD-1 with PD-L1 leads to the activation of the active inhibitory pathway of T-cells, thereby triggering apoptotic conditions and reducing the cell survival rate.[12,35,36]

Regarding adverse inhibitory conditions, the use of CAR-T-cells, which are essentially T lymphocytes, is highly challenging in cancer treatment. The TME has acidic, hypoxic conditions and high levels of oxidative stress substances, which trigger the production of inhibitory immune molecules (PD-1 and CTLA4) and inhibitory immune cells (Tregs). This reduces the invasion and infiltration ability of immune cells. Additionally, the presence of excess PD-1 and CTLA4 inhibits the activation of T-cells. As a result, tumor cells are easily released without degradation by immune cells.[37]

The Role of PD-1/CD28 in the Immunostimulatory Mechanism of CAR-T-cells in Colorectal Cancer

Various techniques have been tested to overcome the inhibitory effects of PD-1 immune checkpoint inhibitors, starting with the use of anti-CD19 and systemic antibodies (nivolumab or pembrolizumab). However, their implementation resulted in various side effects related to excessive immune activity. Therefore, a component that is more specific and safe is needed to inhibit PD-1/PD-L1 activity.[38,39]

A new approach involving the use of an IFP in the form of the CD28 domain can inhibit the PD-1/PD-L1 inhibitory pathway. CD28 is located in the intracellular domain of extracellular PD-1, which functions as a secondary signaling pathway stimulator. This suppresses the activity of inhibitory proteins in T-cells and activates costimulatory pathways when PD-1/PD-L1 interacts.[40] The implementation of PD-1/CD28 on TRuC-T-cells that target tumor cells also increases the production of the cytokines IFN-γ and IL-2 in the TME.[41] On the other hand, the use of PD-L1 on CAR-T-cells increases the sensitivity of the contact area with PD-L1 to the tumor cell surface such that the T-cell activation response increases further.[5]

An increase in T-cell activity due to PD-1/CD28-type IFPs is supported by several studies on leukemia, lymphoma, and even solid cancer.[5,42-44] An *in vivo* leukemia study involving the administration of PD-1/CD28 CAR-T-cells revealed an increase in leukemic clearance and survival of NSG mice. The results revealed that compared to the mice administered CAR-T-cell therapy alone, mice with central and peripheral T-cell modulation (IFP modulation) had a longer life expectancy of 90 days.[5] A trial of PD-1/CD28 CAR-T-cells in 17 PD-L1-positive B-cell lymphoma patients reported a good response. In total, 10 patients experienced an objective response to treatment, and seven others experienced complete remission from their lymphoma. Additionally, no signs of neurological toxicity or CRS were found in the patient.[9] Application in solid cancer was also performed by introducing IFP PD-1/CD28 TRuC-T-cells into NSG mice with inoculation of pancreatic cancer target cell lines (SUIT-MSLN and mesothelioma (MSTO-MSLN). The results revealed smaller tumors (20 mm^3) with TRuC-T PD-1/CD28 treatment than with TRuC-T treatment alone. Moreover, in MSTO-MSLN mice, no significant difference was found in changes in tumor size between TRuC-T PD-1/CD28 and TRuC-T alone.[41] These findings indicated that the use of CAR-T-cells with PD-1/CD28 can increase tumor cell eradication ability, treatment response, and patient survival rates. Studies on PD-1/CD28 integration are listed in Table 2.

Mechanism of Genetic Modification of EcN 1917 and Construction of PD-1/CD28-Integrated CAR-T-cells

Genetic modification of EcN begins by combining an AS component consisting of an sfGFP homodimer that binds to the CAR receptor, linker, and PlGF-2. These components are then incorporated into a plasmid and inserted into EcN. The EcN strain is also equipped with a SLIC system.[6,29] After EcN is modified, the bacteria are cultured and then administered.[6]

The generation of PD-1/CD28 CAR-T-cells begins with sampling normal T-cells from patients through leukapheresis.[12] Next, a CAR component, consisting of specific sfGFP nanobodies, immunoglobulin G4 (IgG4), a CD28 transmembrane domain, and a CD3ζ intracellular domain, is created. Then, the gene for the CAR receptor is cloned and processed into a lentiviral vector that carries the CAR receptor gene. This lentiviral vector is then transduced into the normal T-cells of the patient to obtain CAR-T-cells. The cells are then cultured for expansion before they can be used.[6]

The PD-1/CD28-integrated CAR-T-cells can be constructed using additional lentiviral vectors. PD-1/CD28 receptor generation, according to Liu et al.,[9] involves combining pieces of the extracellular domain of PD-1 and pieces of the transmembrane and intracellular domains of CD28 in the mouse stem cell virus promoter, which is then cloned and inserted into a lentivirus transgenic transcription vector. Lesch et al.[41] showed that the simultaneous transduction of two lentiviral vectors into normal T-cells did not interfere with CAR expression or PD-1/CD28 expression. This study also showed that 58.3% of T-cells successfully expressed CAR and PD-1/CD28 after being transduced simultaneously with two different lentiviral vectors (Figure 3).

Administration of a Synthetic Antigen-Encoded EcN 1917 Probiotic

Intravenous injection of EcN provides an opportunity for EcN to be released systemically and colonize tumors. The main challenge that needs to be overcome in the intravenous route involves determining how to transport EcN to the tumor site before it is eliminated by the reticuloendothelial system. Cao et al.[45] coated EcN with erythrocyte membranes for intravenous administration. They found that retention of EcN increased in the blood and reported a nearly fivefold greater yield on imaging compared to the yield of EcN without erythrocyte membranes. In addition, analysis of the immune inflammatory response by checking serum levels of IL-6, IL-10, and TNF-α, found higher levels in EcN without erythrocyte membrane coating. From this result, the study conclude erythrocyte coating membrane has an effect to preserving EcN bioavailability from natural human inflammatory immune response.[45]

Colorectal cancer in the gastrointestinal tract provides an opportunity for EcN to be administered orally. Gurbatri et al.[26] administered EcN orally to orthotopic colorectal cancer (MSS and MSI) mouse models and found that EcN accumulated in colorectal cancer tissue (~108 CFU/g). The level of EcN accumulated in colorectal cancer tissue was significantly higher than that accumulated in the normal colon (~106 CFU/g) and other organs, such as the liver and spleen (0 CFU/g) (normal colon, liver, and spleen: $p<0.0001$). The main challenge in the oral administration of EcN involves ensuring

TABLE 2: Studies of PD-1/CD28 on CAR-T-cells.

CAR-T cell type	Subject	Dose	Period	Results
Mesothelin-specific CAR-T-cells	NSG mice with SUIT-2 pancreatic cancer cell line xenograft	1×107 CFU	Injection only on the first day	In *in vitro* studies, there was a significant increase in IFN-γ and IL-2 by CAR-T-cells with PD-1/CD28 compared to CAR-T-cells without PD-1/CD28. (IFN-γ, IL-2=$p<0.001$).[41] In *in vivo* studies, CAR-T-cells with PD-1/CD28 were able to significantly inhibit tumor growth compared to regular CAR-T ($p=0.05$).[41]
CD-19, mesothelin, and PSCA specific CAR-T-cells	Mice with EMMESO, PC3-PSCA-PD-L1, and PC3-PSCA cancer cell line xenografts	2×107 CFU	Injection only on the first day	There was a significant inhibition of tumor size by CAR-T-cells with PD-1/CD28 compared with regular CAR-T-cells (SS1BBz/PD1CD28= $p<0.05$; PSCABBz/PD1CD28= $p<0.05$).[42]
CD-19 specific CAR-T-cells	17 patients with PD-L1+ B-cell lymphoma	0.5×106-4×106 CFU/kg	Injection only on the first day	The results of the study on 17 patients showed that 10 out of 17 had an objective response and 7 out of 10 had a complete response within 3 months. Some of the adverse events most frequently experienced by patients were granulocytopenia (100%), pyrexia (100%), anemia (76.47%), thrombocytopenia (70.59%), hypotension (41.17%), CRS Grade 1 (47.06%), and CRS Grade 2 (41.18%). No patient experienced symptoms of neurotoxicity.[9]

CAR-T: Chimeric antigen receptor T; IFN: Interferon; IL: Interleukin.

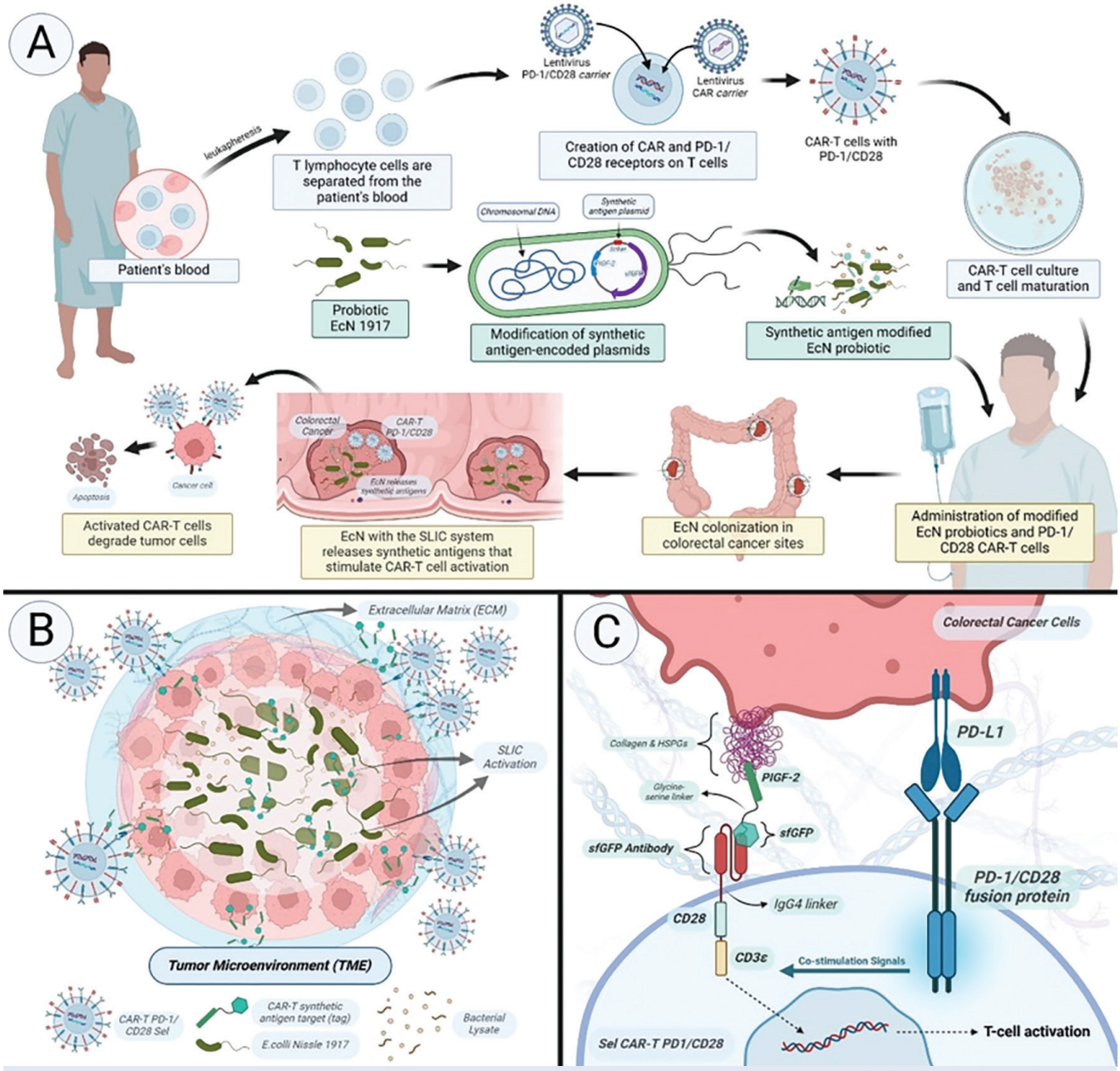

FIGURE 3. Overall mechanism of PD-1/CD28 CAR-T-cells.[6,8,9,33,40-42]

A) Mechanism of construction and administration of PD-1/CD28 integrated CAR-T-cells in colorectal cancer; B) Activation of PD-1/CD28 CAR-T-cells against synthetic antigens produced by EcN via SLIC; C) Structure of synthetic antigen, CAR-T receptor, and PD-1/CD28.

CAR-T: Chimeric antigen receptor T.

that EcN reaches tumor sites that are influenced by various factors, such as gastric acid. One strategy to solve this problem is to use a double-layer polysaccharide hydrogel. The imaging results revealed an increase in the retention of encapsulated probiotics even after 48 h of treatment compared to that of non-encapsulated probiotics, which lasted only 4 h. This occurred due to the nature of the double-layer hydrogel, which completely disintegrated upon reaching the colon.[46] Based on these facts, double-layer hydrogel encapsulation is optimal for the oral administration of EcN.

CONCLUSION

The combination of CAR-T-cell immunotherapy with PD-1/CD28 has promising potential as a new therapeutic option for treating colorectal cancer. Immunotherapy based on

sfGFP-PlGF-targeting CAR-T-cells has a lytic cytotoxic effect specifically on cancer cells and can effectively suppress the formation and growth of tumor cells. The effectiveness of CAR-T-cells can be inhibited by PD-1 molecules in the TME of colorectal cancer. The use of PD-1/CD28 can increase the activity of CAR-T-cells. Through more specific and personalized integrated sfGFP-PlGF PD-1/CD28 CAR-T-cell therapy, a new, more effective method for treating colorectal cancer can be obtained.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.M.W., Concept: T.R.A., Design: T.R.A., Data Collection or Processing: I.M.D., Analysis or Interpretation: A.B., Literature Search: I.M.D., Writing: A.B., Critical Review: B.G.d.L.

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CASE REPORT

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Neurotoxicity in the Era of Immune Checkpoint Inhibitors: A Case-Based Approach

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ABSTRACT

Immune checkpoint inhibitors (ICIs) have transformed the field of cancer immunotherapy and led to substantial improvements in patient outcomes across various malignancies. Neurological toxicities arising from ICI treatment represent a heterogeneous group of complications that manifest across a broad spectrum, ranging from mild symptoms to life-threatening conditions. The present article reviews patients receiving ICI treatment and identifies neurological adverse events observed across all ICI therapeutic modalities. Data were retrospectively evaluated from 500 cancer patients who received immunotherapy treatment between 2020-2022 at Koç University Hospital Medical Oncology Outpatient Clinic. Eight patients (1.6%) who developed immunotherapy-related neurologic side effects were included in the analysis. Demographic and clinicopathologic characteristics, along with laboratory results, were extracted from the medical oncology outpatient clinic database. In this study, 89% (7/8) of the patients were male, with a median age of 59 years (range 44-79). The most common cancer types observed were small cell lung cancer (n=2) and renal cell carcinoma (n=2). A case study is also presented of a patient who developed neurotoxicity following immunotherapy. Immunotherapy emergence has marked substantial advancement in cancer treatment approaches, although neurological side effects require close monitoring. Recognition of diverse neurological complications associated with ICIs and their potential severity remains essential for clinical practice.

Keywords: Immune related adverse events; immune checkpoint inhibitors; immunotherapy; neurological adverse events; neurological toxicities

INTRODUCTION

Immune checkpoint inhibitors (ICIs) have transformed the field of cancer immunotherapy and led to substantial improvements in patient outcomes across various malignancies. These inhibitors, targeting programmed cell death protein 1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), demonstrate efficacy in treating multiple cancers, including melanoma, non-small cell lung cancer, and renal cell carcinoma.[1-3] However, as the clinical use of ICIs has expanded, an increasing number of immune-related adverse events (irAEs) have been reported, including a variety of neurological complications.[4-6]

Neurological toxicities arising from ICI treatment represent a heterogeneous group of complications that manifest across a broad spectrum, ranging from mild symptoms to life-threatening conditions.[1,4,7] Such toxicities affect both central and peripheral nervous systems, manifesting as encephalitis, myasthenia gravis (MG), Guillain-Barré syndrome (GBS), and additional neurological syndromes.[2,5,8] The median onset time for immunotherapy-related neurotoxicity is established at four weeks, with occurrence possible between one week to 68 weeks.[9] While neurological irAEs exhibit lower incidence compared to other irAEs, these events potentially result in significant morbidity and mortality.[3,6,9]

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The precise mechanisms underlying neurological irAE development remain incompletely understood, though attribution to immune system dysregulation caused by checkpoint inhibition has been proposed.[7] Considering the expanding utilization of ICIs in cancer treatment, understanding clinical manifestations, diagnostic approaches, and management strategies for neurological complications becomes essential for clinicians and researchers.[1,7,8]

The present article reviews patients receiving ICI treatment who developed neurological adverse events. Clinical spectrum, treatment approaches, and outcomes of ICI-related neurotoxicity are presented through a case-based methodology.

METHODS

Data were retrospectively evaluated from 500 cancer patients who received immunotherapy treatment between 2020-2022 at Koç University Hospital Medical Oncology Outpatient Clinic. Eight patients (1.6%) who developed immunotherapy-related neurologic side effects were included in the analysis. Demographic characteristics, clinicopathologic features, and laboratory results were extracted from the medical oncology outpatient clinic database.

Consent to Participate

Patient data were obtained retrospectively from medical records following acquisition of written informed consent from patients or designated relatives.

RESULTS

The study population comprised 89% (7/8) male patients, with a median age of 59 years (range: 44-79). Small cell lung cancer (n=2) and renal cell carcinoma (n=2) represented the most frequent cancer types. Cranial radiotherapy was administered to two patients with brain metastases. Atezolizumab treatment was received by four patients (50%). The median time to side effect onset was documented at 10.5 weeks (range: 1-95 weeks). Disease progression or infection resulted in mortality for five patients (63%) during the follow-up period. Clinical data are presented in Table 1.

CASE REPORT

A 59-year-old male patient was diagnosed with laryngeal cancer in February 2021. After completing 28 days of radiotherapy, a salvage laryngectomy was performed in January 2022 due to a local recurrence. Postoperative pathology indicated T4N0M0, and follow-up assessments were scheduled every three months. In July 2022, chemotherapy was initiated for a recurrent lesion that was considered unsuitable for surgical resection. Given a 60% PD-L1 expression level, treatment with a combination of cisplatin, 5-FU, and pembrolizumab was planned.

One month after completing two treatment cycles, the patient presented to the emergency department with right leg weakness, left arm weakness, and ptosis in the right eye. Neurological assessment revealed multiple cranial nerve paralysis manifesting as decreased eye squeezing, rightward tongue deviation and reduced tongue movements. Motor examination demonstrated extremity weakness (proximal more prominent than distal) with bilaterally absent deep tendon reflexes and plantar skin responses. Cranial and cervical magnetic resonance imaging (MRI) with electromyography examination suggested acute disseminated polyneuroradiculopathy (Figure 1).

Admission to neurology service followed with preliminary diagnosis of acute disseminated polyneuroradiculopathy, and intravenous immunoglobulin (IVIG) treatment was administered at 26.8 g over five days. Lumbar puncture

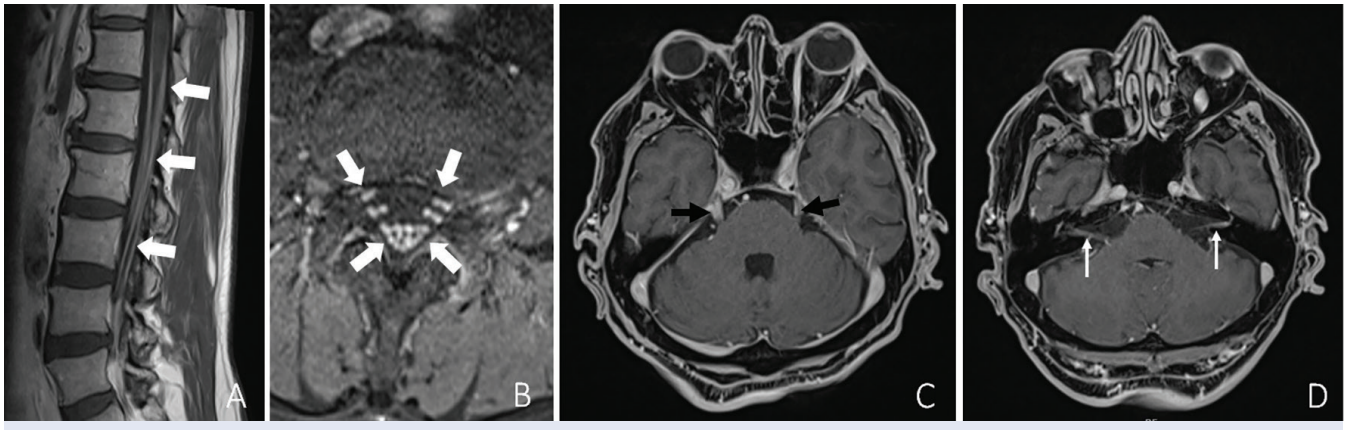

FIGURE 1. A-B) Sagittal plane (A) and axial plane (B) post-contrast T1 weighted images showed diffuse contrast enhancement of cauda equina fibers (thick arrow). C-D: Axial plane post-contrast vibe images demonstrated enhancement of trigeminal nerve (C-black arrow) and vestibular nerve (D-thin arrow).

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TABLE 1: Demographic and clinical findings, treatment characteristics, and outcomes.

Patients	Diagnosis	Treatment	Immunotherapy	Toxicity	Treatment	Status
44 years old female	Breast cancer	IO+ChT	Atezolizumab	Transverse myelitis	Oral methylprednisolone 1 mg/kg/day for 5 days and then oral 60 mg/day maintance - the steroid dose was tapered by 8 mg every five days. During the follow-up period, the dose was reduced to 16 mg/day and continued as maintenance therapy	Symptoms did not improve. The patient passed away due to infection
44 years old male	SCLC	IO+ChT	Atezolizumab	Demyelinating disease	IV methylprednisolone 1000 mg/day for 7 days and then oral steroid 60 mg/day maintance. The steroid dose was tapered by 8 mg every five days. During the follow-up period, the dose was reduced to 8 mg/day and continued as maintenance therapy	Slight improvement in symptoms. The patient passed away due to disease progression
68 years old male	Renal cell carcinoma	IO	Nivolumab	Encephalopathy	Oral methylprednisolone 1 mg/kg/day for 5 days and then oral 60 mg/day maintance- Steroid treatment was tapered by 12 mg/day every 5 days and discontinued within a month	Slight improvement in symptoms. The patient passed away due to aspiration pneumonia
61 years old male	Malignant melanoma	IO+IO	Nivolumab plus Ipilimumab	Encephalopathy	IV methylprednisolone 1 mg/kg/day for 5 days and then oral 60 mg/day maintance +IVIG 33gr, D1-4. Steroid treatment was tapered by 12 mg/day every 5 days and discontinued within a month	Symptoms improved, and steroid dosage was tapered off and eventually discontinued. The patient continued with single-agent IO treatment.
57 years old male	SCLC	IO+ChT	Atezolizumab	Encephalopathy	Oral methylprednisolone was administered at 16 mg/day for five days, after which the dose was reduced by 4 mg every three days, with the treatment discontinued within three weeks	Symptoms improved, and the steroid dosage was tapered off and eventually discontinued. Not avaliable follow-up data
58 years old male	Larynx tumor	IO+Cht	Pembrolizumab	Acute disseminated polineuroradiculopaty with miyasthenic syndrome	Oral steroid 30 mg/day + IVIG 27 gr, D 1-5 and then iv pulse metyl prednisone 250 mg/day/five days, pyridostigmine 4x60 mg. The prednisolone dose was reduced to 30 mg and and continued as maintenance therapy	Symptoms improved, and the steroid dosage was tapered off but not discontinued. The patient passed away due to disease progression
79 years old male	Renal cell carcinoma	IO	Nivolumab	Encephalopathy	Oral methylprednisolone was administered at 16 mg/day for five days, after which the dose was reduced by 4 mg every three days, with the treatment discontinued within three weeks	Alive -Not avaliable follow-up data
68 years old male	Lung adenocarcinoma	IO+ChT	Atezolizumab	Demyelinating disease	Intravenous (IV) methylprednisolone was administered at 1000 mg/day for 5 days, followed by oral steroids at a maintenance dose of 60 mg/day. IVIG, at a total dose of 27 g, was administered over days 1-4. The steroid dose was tapered by 8 mg every five days. During the follow-up period, the dose was reduced to 16 mg/day and continued as maintenance therapy	Symptoms did not improve. The patient passed away due to infection

SCLC: Small cell carcinoma; ChT: Chemotherapy; IO: Immunotherapy; IVIG: Intravenous immunglobuline.

revealed albuminocytological dissociation, while paraneoplastic panel, autoimmune encephalitis panel, meningitis panel, *Mycobacterium tuberculosis* polymerase chain reaction, and ganglioside panel yielded negative results. Clinical findings showed no improvement with IVIG and 30 mg prednisolone. Consequently, pulse steroid therapy using methylprednisolone 250 mg was initiated for five additional days. Mild neurologic symptom improvement was observed under corticosteroid therapy. Pyridostigmine treatment 1×60 mg was initiated due to persistent right eye ptosis and positive anti-acetylcholine receptor antibody (1.07). Final diagnosis indicated combined disseminated polyneuroradiculopathy with myasthenic syndrome. Prednisolone and pyridostigmine 4×60 mg treatment was continued.

Under combined prednisolone 60 mg and pyridostigmine 4×60 mg therapy, significant improvement in neurological findings was observed. Discontinuation of pembrolizumab treatment occurred, with subsequent initiation of carboplatin, paclitaxel, and cetuximab therapy. The prednisolone dose was reduced to 30 mg during follow-up, while pyridostigmine 4×60 mg treatment continued, leading to complete neurological recovery. Unfortunately, the patient passed away due to disease progression three months later.

DISCUSSION

Immunotherapy has become a groundbreaking cancer treatment approach, focusing on immune checkpoints and leveraging the immune system to target tumor cells. Although these therapies have shown notable therapeutic benefits, they are linked to a spectrum of irAEs, including neurological toxicities.[1] These irAEs can present as diverse neurological complications, highlighting the need to understand their incidence, pathophysiology, and management in clinical practice. The incidence of neurological complications associated with ICIs varies, with reported rates ranging from 0.1% to 6%.[3] Research by Larkin et al.[2] reported neurologic serious adverse events in 6.1% of patients receiving nivolumab combined with ipilimumab and in 2.7% of patients treated with nivolumab alone. The range of ICI-related neurological complications includes encephalitis, meningitis, myelitis, demyelinating neuropathies such as GBS, and MG, among others.[4,5]

The exact pathophysiological mechanisms behind ICI-associated neurological toxicities are not fully understood, but it is believed that these toxicities may arise from a dysregulated immune response, resulting in autoimmune or inflammatory processes.[1,6] Case studies and cohort analyses suggest that humoral immune responses, such as the presence of neuromuscular and brain-reactive autoantibodies, may play a role in the onset of irAEs. Notably, patients with irAEs have shown a higher prevalence of neuromuscular autoantibodies compared to those without such events. Molecular mimicry may also contribute to the variability in irAEs across cancer types, potentially due to shared expression of gangliosides between melanoma cells and Schwann cells, which form myelin around peripheral nerves. This hypothesis may help explain the increased neurotoxicity observed in certain melanoma patients.[10,11] For instance, a case report highlighted a melanoma patient who developed autoimmune encephalitis linked to ICI therapy.[8]

A thorough assessment of the patient's neurological symptoms is essential, with potential symptoms including headaches, muscle weakness, altered consciousness, and seizures. A critical aspect of diagnosis is evaluating whether the onset of neurological symptoms correlates with the timing of ICI therapy. The severity and scope of neurological involvement can be assessed through diagnostic methods such as MRI, electroencephalography, and cerebrospinal fluid analysis. It is also crucial to exclude other potential causes, including infections, cerebrovascular incidents (e.g., ischemia or hemorrhage), paraneoplastic syndromes, and cranial metastases. Diagnosing neurological adverse events requires a comprehensive evaluation, and if myocarditis is suspected, further testing-including electrocardiogram, troponin levels, brain natriuretic peptide, CK-MB, cardiac ultrasound, and cardiac MRI-is recommended. Additionally, pulmonary function tests and video fluoroscopic swallowing studies can help assess restrictive syndromes and dysphagia associated with MG, myositis, or GBS. Although rarely required, a biopsy may be considered in cases where there is a need to exclude alternative diagnoses, such as chronic pachymeningitis or persistent concerns of leptomeningeal carcinomatosis, even if a lumbar puncture is negative.[12,13]

Management of ICI-related neurological side effects generally involves discontinuing immunotherapy and initiating corticosteroid treatment.[7] In certain cases, additional immunosuppressive therapies, such as IVIG or plasmapheresis, may be necessary.[5] For Grade 2 immunotherapy-associated MG, pyridostigmine combined with prednisone at an oral dose of 0.5 mg/kg/day (or an equivalent) is recommended, with dosage tapering based on symptom improvement. For Grade 3-4 toxicity, IVIG at a total dose of 2 g/kg over five days or plasmapheresis is advised.[9] For patients unresponsive to initial treatment, second-line immunosuppressive therapies like rituximab may be considered.[14] Early detection and appropriate management of these neurological irAEs are crucial to prevent permanent neurological impairment and enhance patient outcomes.[15]

CONCLUSION

In conclusion, although immunotherapy has brought significant advancements in cancer treatment, awareness of its potential neurological side effects remains essential. Clinicians must be vigilant regarding the broad spectrum of neurological complications associated with ICIs and their varying levels of severity. Prompt identification and effective management of these adverse events are vital to reducing morbidity and optimizing patient care. Further research is needed to elucidate the pathophysiology of these complications and to develop more targeted management strategies.

Ethics

Informed Consent: Patient data were obtained retrospectively from medical records following acquisition of written informed consent from patients or designated relatives.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Ö.A., Y.E.A., F.S., Concept: Ö.A., Y.E.A., F.S., Design: Ö.A., Y.E.A., F.S., Data Collection or Processing: Y.E.A., B.B.K., A.D., B.K., S.A., Ş.L., Analysis or Interpretation: Ö.A., Y.E.A., F.S., M.S.A., Literature Search: A.D., P.O., Ö.A., F.S., Writing: Ö.A., Y.E.A., M.C.B., O.Ç., Critical Review: P.O., F.S., N.M.M.

Conflict of Interest: No conflict of interest was declared by the authors.

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CASE REPORT

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Is There Any Relationship between Bilateral Pleural Effusion and Blast Crisis in Chronic Myeloid Leukemia?

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ABSTRACT

Pleural effusion in chronic myeloid leukemia (CML) has been rarely reported in medical practice. Additionally, the correlation of pleural effusion with blast crisis is unknown. This study reported a 66-year-old male patient who was diagnosed with bilateral pleural effusion three months before the blast transformation. Bilateral pleural effusion spontaneously resolved before the blast transformation. The cause of pleural effusion was unknown. Bilateral pleural effusion caused by unknown or known factors, except for that induced by drugs, is a poor prognostic marker for CML and is an unusual indicator of blast crisis. The presentation of the study case indicates that bilateral pleural effusion is an atypical and discernible early indicator of blast crisis onset in patients with CML.

Keywords: Pleural effusion; chronic myeloid leukemia; blastic transformation

INTRODUCTION

Pleural or peritoneal involvement is rare in leukemias. In contrast, pleural or peritoneal involvement is frequently observed in solid hematological cancers and lymphomas. Pleural effusion may occur in patients with chronic myeloid leukemia (CML).[1,2] The etiological factors for pleural effusion in patients with CML include infections, hypoproteinemia, blast involvement, extramedullary (spleen, lymph nodes, skin, meninges, and bone) hematopoiesis, pleural capillary obstruction, and drugs.[3] Here, we report a case with unusual pleural effusion that manifested three months before the blast crisis of CML. This case report aimed to demonstrate the potential prognostic value of bilateral pleural effusion preceding blast crisis and improve our understanding of this clinical manifestation in CML.

CASE REPORT

The patient was a 66-year-old male who was initially diagnosed with CML in 1999. An accidental blood test revealed a high leukocyte count when the patient was aged 49 years. Fluorescent *in situ* hybridization (FISH) analysis revealed that the patient tested positive for BCR-ABL mutation. The patient was initially treated with interferon-alpha. After 3 years, the interferon dosage was reduced to 3 million units due to elevated liver enzymes. Interferon treatment was continued until 2007 because of the complete cytogenetic response. The patient was determined to be in complete cytogenetic remission owing to negative FISH results since the beginning of interferon-alpha therapy. The treatment was changed to imatinib mesylate (400 mg per day) in 2008 owing to the upregulation of liver enzymes and blood lipids. The patient tested negative in FISH tests up to 2016 and was in complete cytogenetic remission.

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In June 2016, the patient was admitted to the hospital with the complaint of left-side pain and the following presentations: fever: 36.5 °C; heart rate: 75/min; blood pressure: 123/70 mmHg. The complete blood count revealed the following findings: hemoglobin: 14.4 g/dL; platelet count: 117,000 platelets/mm^3; leukocyte count: 6,650 leukocytes/mm^3. The peripheral blood smear examination was unremarkable. The erythrocyte sedimentation rate was 75 mm/hour. The patient exhibited physiological biochemical parameters. Antibiotic treatment initiated at the hospital did not alleviate the complaints of the patient. Analysis of tuberculosis-causing agents and other bacterial and viral infectious agents did not yield positive results. In the thorax computed tomography scan, pleural effusion was detected on both hemithoraces (9 mm on the right and 30 mm on the left).

No evidence of heart failure was noted. Pleural effusion examination [protein, lactate dehydrogenase (LDH), cell count, differential, and cytology] did not reveal leukemic involvement or other causes. The patient was discharged in August as the complaints improved and the pleural effusion disappeared although weakness persisted (Figure 1).

In October 2016, the patient was hospitalized again with complaints of high fever and fatigue. The blood count revealed pancytopenia (leukocyte count: 2,200 leukocytes/mm^3; platelet count: 18×10^3 platelets/mm^3; hemoglobin: 10 g/dL). Bone marrow biopsy revealed the infiltration of myeloid blast cells.

Morphological and immunohistochemical findings were consistent with the transformation of CML into acute myeloid leukemia (Figure 2). After 1 week, the patient died due to tumor lysis syndrome and acute renal insufficiency (creatinine: 9.1 mg/dL; LDH: 22.970 IU; potassium: 5.5 mmol/L; urea: 389 mg/dL; uric acid: 13.8; Ca^{2+}: 8 mg/dL) without leukemia treatment. Informed consent was obtained by the daughter of the patient to publish this case report.

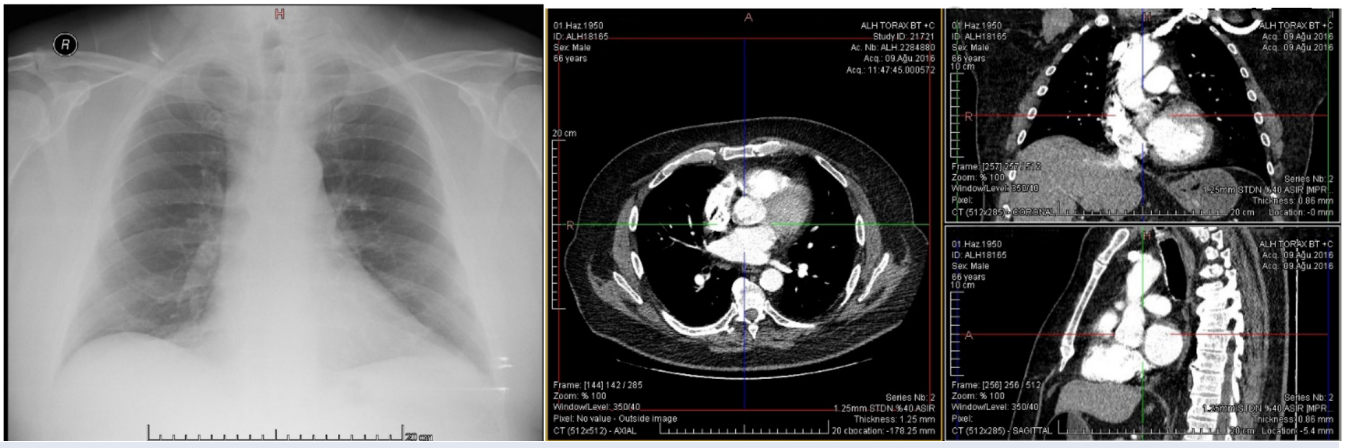

FIGURE 1: Computed tomography scan of the thorax revealed that pleural effusion spontaneously resolved after three months.

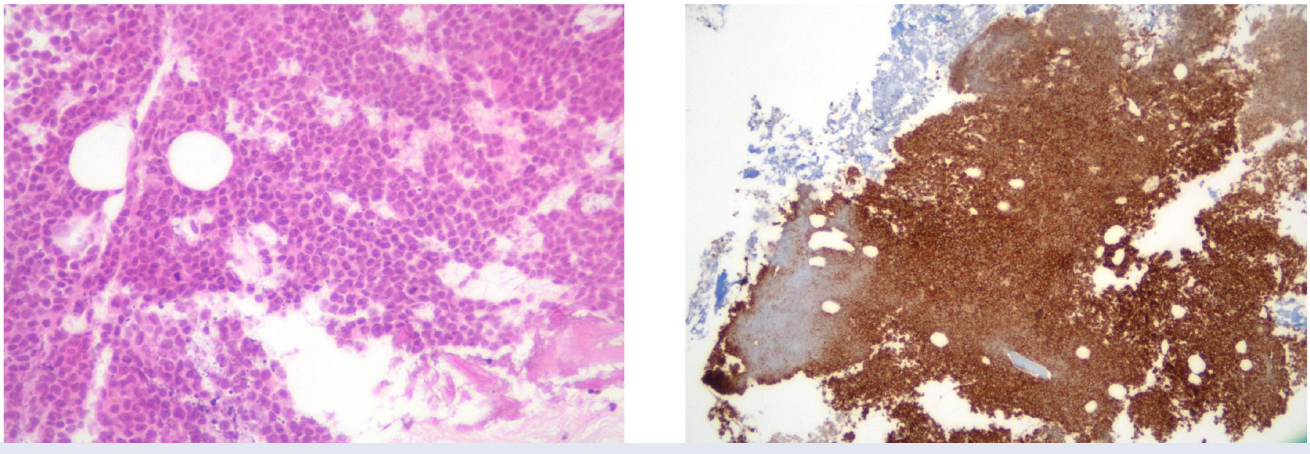

FIGURE 2: Diffuse myeloid blast cell infiltration in the bone marrow (H&E, ×100). IHC analysis of CD34$^+$ blast cells (magnification: ×100).

IHC: Immunohistochemistry, H&E: Hematoxylin and eosin

DISCUSSION

Pleural effusion is a rare finding in both the chronic phase and the acute blast phase of CML. The etiological factors of pleural effusion include leukemic infiltration, extramedullary hematopoiesis, infection, hypoproteinemia, pleural capillary obstruction, leukemic infiltration of the interstitial tissue, and drugs.

The infiltration of leukemia into the pleura typically occurs at the same time as or shortly before the blast crisis phase of bone marrow development.[4] The most common infiltration sites are the brain, testis, skin, breast, soft tissue, synovium, lymph nodes, bones, and the nervous system. However, leukemic infiltration has also been reported in the gastrointestinal tract, ovaries, kidneys, and pleura. Pleural involvement is rare. Isolated pleural blast crises without medullary change are extremely uncommon.[5] In the study patient, pleural blast infiltration was not observed in the chronic and blast phases.

Extramedullary hematopoiesis is also a potential cause of pleural effusion in CML. In contrast to pleural leukemic infiltration, extramedullary hematopoiesis involves hematopoietic cells of the erythroid, myeloid, and megakaryocytic types. The study case did not exhibit extramedullary hematopoiesis.

Infection and hypoproteinemia are proposed as non-cancerous causes of effusion.[2] The study case did not exhibit hypoproteinemia or any other infection.

Cytokine production-induced uncontrolled leukocytosis and enhanced capillary permeability may cause pleural capillary blockage or leukemic cell invasion into the interstitial tissue, leading to the development of pleural effusions in patients with CML.[4] In patients with myeloproliferative disease, the upregulated levels of interleukin (IL)-8, IL-2R, IL-12, IL-15, and IP-10 were independent predictors of poor survival.[6] Similar cytokine profiles have been reported during chimeric antigen receptor T-cell therapy and the infusion of hypercellular leukapheresis products.[7] Leukostasis and platelet dysfunction are predisposing factors for hemorrhagic effusion in CML. The study patient did not exhibit leukocytosis during pleural effusion.

Drugs can also induce pleural effusion in CML. Dasatinib and imatinib, which are tyrosine kinase inhibitors (TKIs), are used to treat CML. TKIs can induce pleural effusion.[8] The pathophysiology of dasatinib-induced pleural effusion has not been elucidated, although TKIs were reported to exert off-target effects on the immune system.[9] The study patient underwent imatinib treatment, but the pleural effusion resolved spontaneously despite the non-cessation of imatinib.

CONCLUSION

Thus, pleural effusion in the study case, which started in June, was resolved in August. One potential reason for pleural effusion is an unknown infectious agent. Unknown causes and cytokines released before blast transformation can also cause pleural effusion. Bilateral pleural effusion caused by known or unknown factors, except that caused by drugs, is a poor prognostic marker in patients with CML and an unusual indicator of blast crisis.

Ethics

Informed Consent: Informed consent was obtained by the daughter of the patient to publish this case report.

Footnotes

Authorship Contributions

Surgical and Medical Practices: E.E., F.A., Concept: E.E., F.A., Design: E.E., F.A., Data Collection or Processing: E.E., M.B.A., M.S., Analysis or Interpretation: E.E., F.A., Literature Search: E.E., F.A., Writing: E.E., M.S., F.A.

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CASE REPORT

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Germline *ATM* Variation in a Young Patient Diagnosed with Breast Cancer Presenting with Vaginal Neuroendocrine Carcinoma

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ABSTRACT

ATM plays a crucial role in repairing DNA damage and maintaining genomic stability. Mutations in *ATM* are associated with increased breast cancer risk and the development of various neuroendocrine carcinomas, including small-cell lung carcinoma and neuroendocrine tumors of the gastrointestinal tract. Here, we present a case with a heterozygous *ATM* variant [NM_000051.4.7174C>T(p.Arg2392Trp)], which is classified as a variant of uncertain significance (VUS) in the ClinVar database. This patient, who was initially treated for breast cancer, was later diagnosed with a rare vaginal neuroendocrine carcinoma at month 31 post-breast cancer diagnosis. In contrast to most cases, the patient tested negative for human papillomavirus (HPV)-DNA. Based on the rare presentation of neuroendocrine carcinoma and the negative HPV-DNA status, we proposed that VUS of *ATM* may be associated with cancer development and has pathogenic roles.

Keywords: *ATM* protein; human; carcinoma; neuroendocrine; breast neoplasms

INTRODUCTION

ATM, a crucial component of the DNA damage response pathway, preserves genomic integrity by facilitating the repair of double-strand DNA breaks. Mutations in *ATM* are reported to contribute to breast cancer development.[1] Individuals with heterozygous or homozygous *ATM* mutations are at an increased risk of developing breast cancer. In particular, the prevalence of these mutations is high in some breast cancer subtypes. Additionally, *ATM* mutations are associated with various neuroendocrine carcinomas, including small-cell lung carcinoma, large-cell neuroendocrine carcinoma, and neuroendocrine tumors of the gastrointestinal tract.[2]

Here, we present a patient with a heterozygous *ATM* variant [NM_000051.4.7174C>T(p.Arg2392Trp)] that is classified as a variant of uncertain significance (VUS) in the ClinVar database. The patient was diagnosed with vaginal neuroendocrine carcinoma, which manifested as vaginal mass and vaginal bleeding, at month 13 post-breast cancer diagnosis. Previous studies and case reports have reported that vaginal neuroendocrine carcinoma, a highly rare condition, is typically associated with human papillomavirus (HPV) infection. However, the study patient tested negative for HPV. We hypothesized that this heterozygous *ATM* variant that is classified as a VUS may have a pathogenic role, potentially contributing to the development of cancer.

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CASE REPORT

A 30-year-old female patient presented with a palpable mass in the right breast. The patient had no known comorbidities and no family history of cancer. Magnetic resonance imaging, which was performed after the physical examination, revealed an axillary mass (16 mm×9 mm) with a malignant appearance. Pathological lymph node involvement was not observed in the axilla. Positron emission tomography/computed tomography (PET/CT) did not reveal distant metastasis. The patient, who was diagnosed with invasive ductal carcinoma via tru-cut biopsy, underwent breast-conserving surgery and a sentinel lymph node biopsy (Figure 1). After the surgery, a mass (13 mm×10 mm×10 mm) with clean surgical margins was excised. Perineural invasion and lymphovascular invasion were not observed in this mass. The molecular characteristics of the mass were as follows: estrogen receptor level, 20%; progesterone receptor level, 5%; ERBB2 status, negative; MKI67 expression level, 80%; tumor grade, grade 3 (Figures 2, 3). Sentinel lymph node biopsy did not reveal metastasis (0/3). The patient was referred to the oncology clinic with the diagnosis of pT1cN0M0 (stage 1A) luminal B invasive ductal carcinoma. Breast risk scoring tests were not performed owing to the lack of health system reimbursement. The histopathological findings were consistent with high-grade tumors (grade-3, MKI67-high). The patient received four cycles of dose-dense anthracycline-cyclophosphamide (doxorubicin=60 mg/m^2 and cyclophosphamide=600 mg/m^2) chemotherapy after genetic consultation. Next, the patient underwent adjuvant radiotherapy without adverse effects. The patient was then initiated on goserelin and tamoxifen treatment as adjuvant hormone therapy. The *ATM* variant [c.7174C>T(p.Arg2392Trp)] was detected in the heterozygous form (Figure 4). This variant was classified as VUS in the ClinVar database (ClinVarID: 186868).

The OncoRisk next-generation sequencing (NGS) panel (comprising 31 genes associated with several hereditary cancer syndromes) was used for the analysis. The panel did not detect any variants, except for the *ATM* variant. Next, *BRCA1* and *BRCA2* were subjected to multiplex ligation-dependent probe amplification analysis, which revealed no deletions or duplications.

The patient underwent follow-up mammography, breast ultrasound, and abdominal ultrasound and received hormone therapy without adverse effects. However, the patient developed a vaginal mass and vaginal bleeding at month 31 post-breast cancer diagnosis. An excisional biopsy of the vaginal mass was performed. The MKI67 score in the vaginal mass was 60%. Additionally, the mass tested negative for mammaglobin, p63, p40, estrogen receptor, progesterone receptor, ERRB2, CK 5/6, GATA-3, and PAX-8 (Figure 5). The nuclei were mostly large and hyperchromatic. The mass tested positive for chromogranin A (Figures 6, 7). The results of the excisional vaginal mass biopsy revealed neuroendocrine carcinoma. PET/CT scanning was performed for staging. Increased ^{18}F-fluorodeoxyglucose (FDG) uptake consistent with primary malignancy was observed in the vagina. Meanwhile, increased ^{18}F-FDG uptake consistent with metastasis was observed in the inguinal, femoral, and pelvic lymph nodes. Additionally, increased FDG uptake consistent with metastasis was observed in the left sixth rib, L1 vertebra, and right pubic bone (Figures 8-11). The patient tested negative for HPV-DNA. The patient was diagnosed with stage 4 metastatic neuroendocrine carcinoma and treated with etoposide, cisplatin, and zoledronic acid. The patient is currently under treatment.

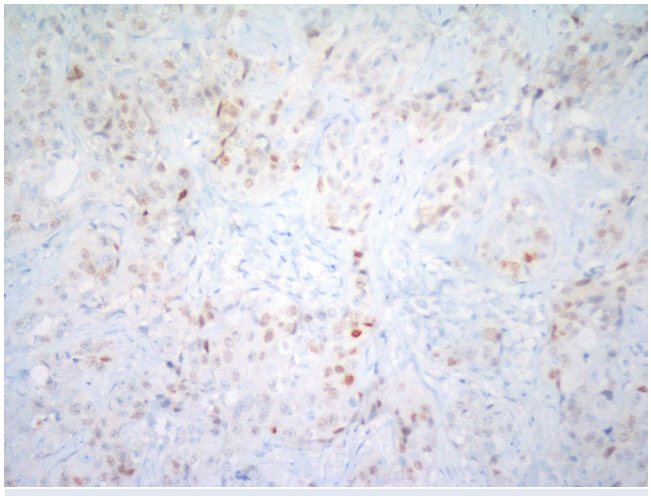

FIGURE 1: Breast cancer-pleomorphic invasive tumor cells adjacent to normal breast ducts (H&E, x10).

H&E: Hematoxylin&eosin

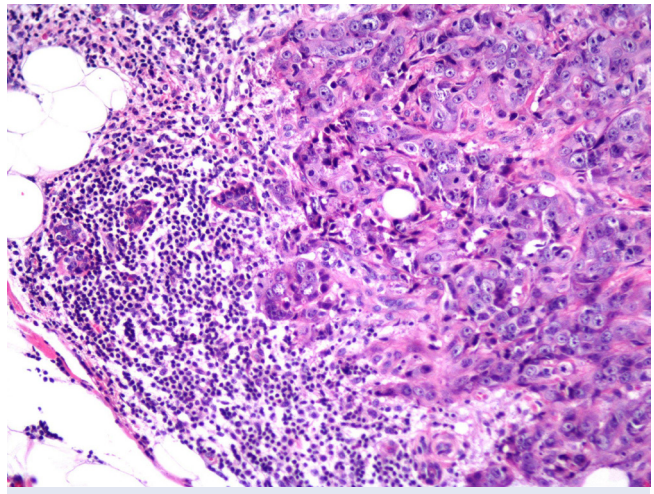

FIGURE 2: Breast cancer-weak ER positivity in tumor cells (DAB, x10).

ER: Estrogen receptor

Informed consent forms were obtained from the patient and the physicians who participated in the study.

DISCUSSION

Pathological *ATM* variants are rare in breast cancer. The pooled prevalence rate of *ATM* variants in patients with breast cancer is reported to be 7%.[3] However, these variants are commonly detected in certain subtypes of breast cancer, such as triple-negative breast cancer.[4] The clinical characteristics of patients with breast cancer exhibiting pathological *ATM* variation are distinct. Previous studies have reported that *ATM* mutation-associated breast cancers are likely to be high-grade tumors, have an increased frequency of *TP53* mutations, and exhibit genomic instability.[4] Based on the clinical implications of pathological *ATM* variation, genetic testing may be recommended for patients with breast cancer, especially for those with a family history of breast or other related cancers.

Neuroendocrine carcinoma of the vagina, a rare malignancy, typically affects postmenopausal women, although it has also been reported in younger individuals.[5-8] This aggressive and high-grade cancer is often diagnosed at an advanced stage.[9] Vaginal cancers account for less than 1-2% of all gynecological cancers, and neuroendocrine carcinoma represents a small percentage of vaginal cancers.[5,6] Previous studies have reported that patients with gyneco-oncological neuroendocrine cancers test positive for HPV-DNA.[9] In addition to the case reported in this study, 28 cases of primary vaginal neuroendocrine carcinoma have been previously reported.[9] The data on *ATM* mutations in neuroendocrine carcinoma are limited as it is a rare cancer.

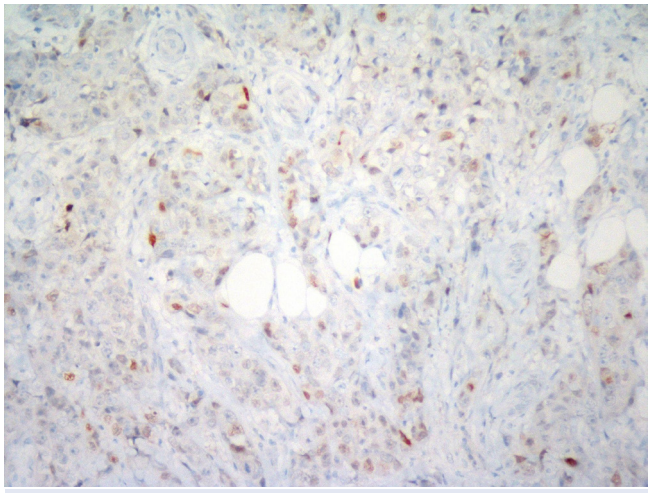

FIGURE 3: Breast cancer-weak PR positivity in tumor cells (DAB, x10).

PR: Progesterone receptor

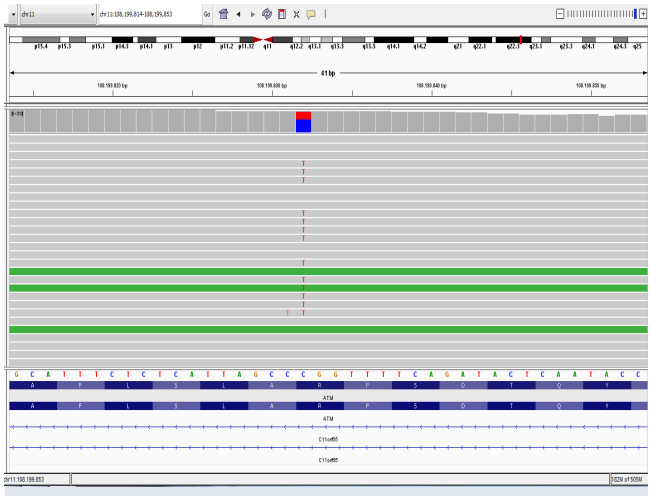

FIGURE 4: Genetical analysis for ATM variation.

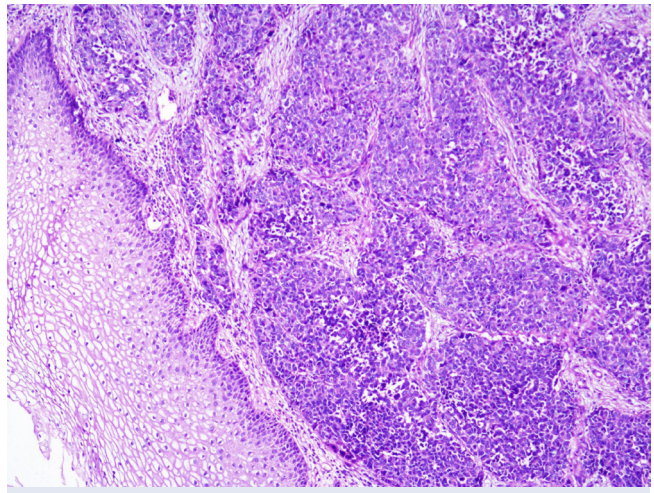

FIGURE 5: Vagen-solid tumor islands under vagen squamous epithelium (H&E, x100).

H&E: Hematoxylin&eosin

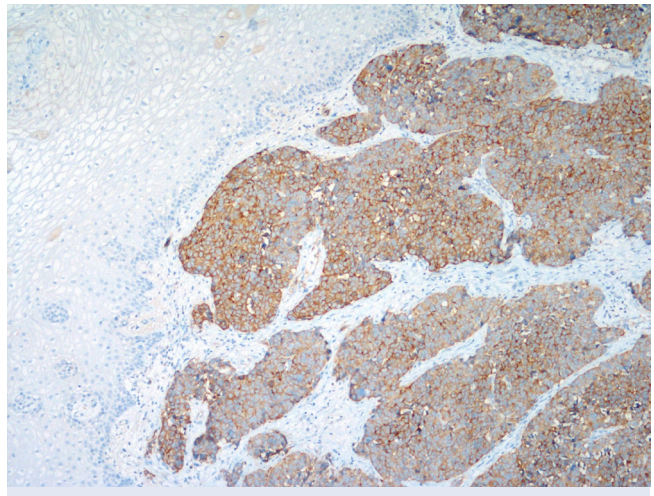

FIGURE 6: Vagen-diffuse synaptophysin positivity in tumor cells (DAB, x100).

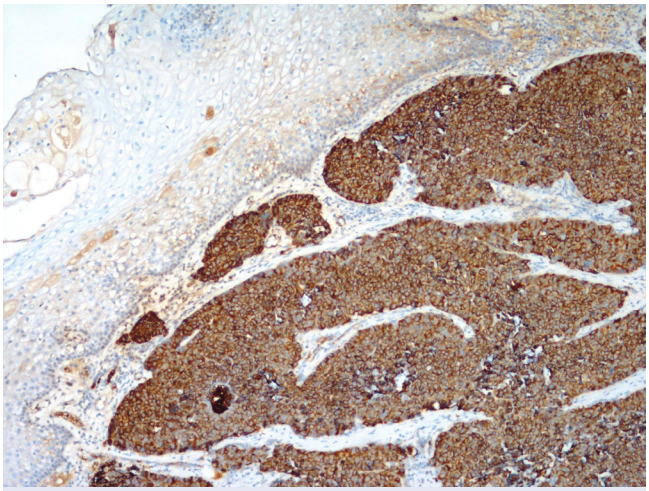

FIGURE 7: Vagen-diffuse chromogranin positivity in tumor cells (DAB, x100).

The *ATM* variant [c.7174C>T(p.Arg2392Trp)] detected in the study patient was classified as VUS in the ClinVar database and as a "possible pathogenic variant" according to the American College of Medical Genetics criteria.

This is the first study to report this variant in neuroendocrine carcinoma. This variant is potentially associated with both breast cancer and neuroendocrine carcinoma.

The study patient tested negative for HPV-DNA, which was in contrast to the HPV-DNA-positive status previously reported in gyneco-oncological neuroendocrine cancers.[10,11] This indicates that this genetic variant is specifically associated with vaginal neuroendocrine carcinoma.

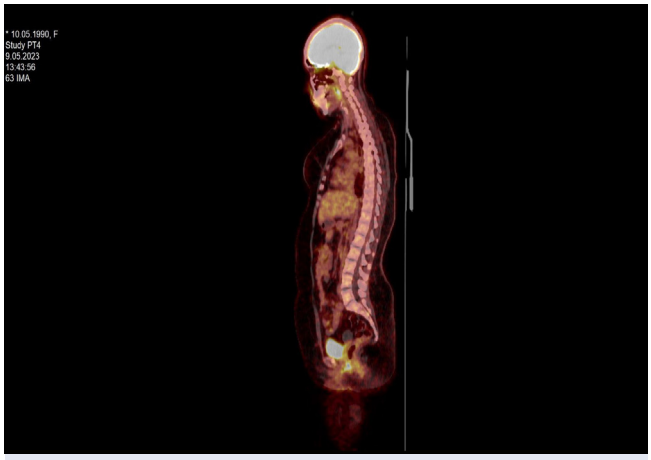

FIGURE 8: Positron emission tomography/computed tomography scan for neuroendocrine carcinoma-1.

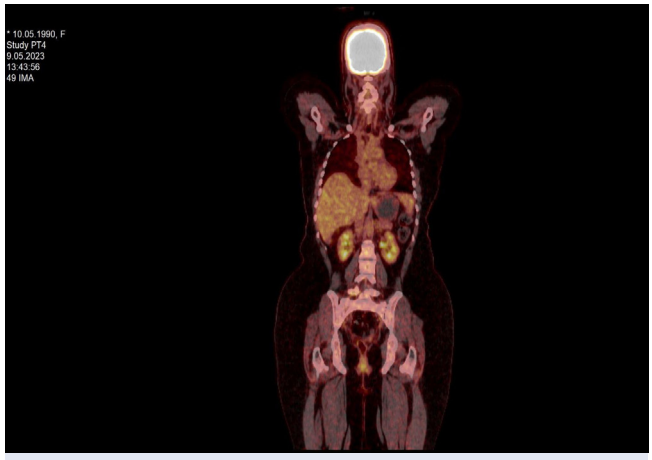

FIGURE 10: Positron emission tomography/computed tomography scan for neuroendocrine carcinoma-3.

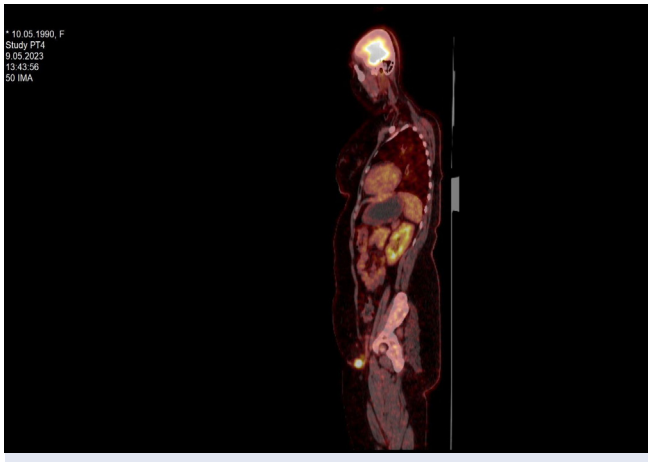

FIGURE 9: Positron emission tomography/computed tomography scan for neuroendocrine carcinoma-2.

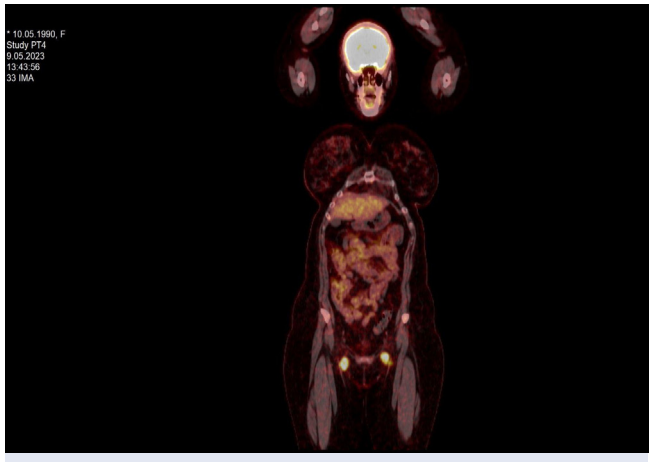

FIGURE 11: Positron emission tomography/computed tomography scan for neuroendocrine carcinoma-4.

CONCLUSION

Limited information is available on the genomic profile of vaginal neuroendocrine cancer based on the NGS panel owing to the rare occurrence of this cancer. Further genetic studies will improve our understanding of the genetic characteristics of vaginal neuroendocrine cancer.

Ethics

Informed Consent: Informed consent forms were obtained from the patient and the physicians who participated in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O.Ü.Ü., S.A., Concept: E.G.K., Design: E.G.K., Data Collection or Processing: E.G.K., E.E.P., Analysis or Interpretation: E.G.K., Literature Search: E.G.K., Writing: E.G.K., Critical Review: T.R.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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CASE REPORT

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Antibody-Drug Conjugates as a Potential a Systemic Treatment Strategy for Brain Metastases in Cases of Solid Tumors

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ABSTRACT

Large molecular antibody-drug conjugates (ADCs) are able to easily access the site of metastasis in the brain due to the edematous structure of brain metastases, facilitating considerably high concentrations of the cytotoxic component of these ADCs in the intracellular and peritumoral environment. Therefore, these ADCs are expected to achieve deeper responses in brain metastases. In this context, the present study discusses the cases of two different patients. The first patient had lung adenocarcinoma with asymptomatic brain metastases, visceral metastases, and bone metastases, and was treated with Sacituzumab govitecan. The second patient had human epidermal growth factor receptor 2-positive breast cancer with lung and brain metastases and received treatment with Trastuzumab deruxtecan. The use of ADC achieved a complete response in brain metastases in both cases, as revealed by the results of cranial magnetic resonance imaging. Accordingly, it is suggested that efficacy evaluations regarding brain metastases should be investigated as a separate secondary endpoint in studies conducted on the use of ADC and that brain metastases should be included as a special research topic in antibody drug design and development.

Keywords: Immunoconjugates; brain neoplasms; brain edema

INTRODUCTION

Brain metastases are common in solid tumors, with lung and breast cancers among the most common cancers with brain metastases. Brain metastases develop in 10-36% of all cases of lung cancer and 10-16% of all cases of breast cancer, affecting the prognosis of these patients negatively.[1-3] The incidence of brain metastasis is particularly higher in the cases of non-small cell lung cancer (NSCLC) with estimated glomerular filtration rate (EGFR) or ALK mutation, with metastasis observed in 50-60% of these patients.[4,5] Median survival in patients of lung cancer with brain metastases ranges from 3 months to 46.8 months, and this variation is attributed to the potent effects of ALK and EGFR inhibitors on brain metastases in eligible NSCLC patients.[6] A meta-analysis of patients with breast cancer revealed that 31% of human epidermal growth factor receptor 2 (HER2)-positive patients, 32% of triple-negative patients, and 15% of hormone receptor-positive and HER2-negative patients with metastatic breast cancer developed brain metastases.[7] Median survival in breast cancer patients with brain metastases is just 14.4 months.[8] The prognosis is worse in triple-negative breast cancer patients with brain metastases, who present a median survival of just 3.4 months, while the corresponding duration is 20.3 months in HER2-positive patients.[9,10] Significant advances have been achieved in the systemic treatment of brain metastases in cases of certain tumors such as ALK and EGFR-positive NSCLC. The cases of brain metastases in most solid tumors, on the other hand, remain to achieve improvements in this regard.

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Available at www.jos.galenos.com.tr

Antibody-drug conjugates (ADCs) are prepared by combining an antibody and a cytotoxic agent developed against an antigen of cancer cells with a strong bond. When the ADCs reach the antigen-expressing tumor cells, cytotoxic molecules are released. After the destruction of the target tumor cells, these cytotoxins are released into the cellular environment, which affects the neighboring tumor cells as well. This phenomenon is referred to as a bystander effect, in which the cells that do not express the antigen are also killed.[11]

Since brain metastases have an edematous structure, large molecular ADCs have easy access to the site of metastasis in the brain. This facilitates reaching considerably high doses of the cytotoxic component in the intracellular and peritumoral environment, causing brain metastases to be exposed to intense cytotoxicity. This mechanism enables achieving deeper responses in brain metastases with the use of ADCs.

In the text ahead, the cases of two patients treated with ADCs are presented in the context stated above.

CASE REPORTS

CASE 1

A 56-year-old woman was admitted to the emergency department when she had an epileptic seizure in July 2021. Mass excision was performed after the cranial magnetic resonance imaging (MRI) results revealed a solitary left temporal mass. The results of pathological analysis were consistent with lung adenocarcinoma. Thorax abdominal computed tomography (CT) revealed a T3Nx mass in the right lung. The patient underwent whole-brain radiotherapy after surgery. The systemic treatments used for the patient and the results achieved are presented in Figure 1.

In November 2022, Sacituzumab govitecan was administered at a dosage of 10 mg/kg on Day 1, and 8 q21 was commenced for this patient with multiple asymptomatic brain metastases, visceral metastases, and bone metastases. Complete response was achieved after 6 weeks, as revealed in the control cranial MRI analysis. Thorax and abdominal CT revealed partial response, and similar findings were obtained in the follow-up (Figure 2).

CASE 2

A 47-year-old woman with ER and PR-negative, HER2-positive invasive breast carcinoma along with *de novo* bone metastases and multiple liver metastases was treated with docetaxel, pertuzumab, Trastuzumab, followed by maintenance treatment with Trastuzumab and pertuzumab. Imaging performed 21 months after the diagnosis revealed a 1.5 cm metastatic nodular lesion in the left lung and a 35*17 mm metastatic mass lesion in the left cerebral hemisphere. Trastuzumab deruxtecan at a dosage of 5.4 mg/kg q21 was commenced. Control imaging after treatment revealed regression of the metastatic lesion in the lung and complete response to treatment in the cranial metastases. The systemic treatments used for the patient and the results achieved are presented in Figure 3.

Figure 4 presents the rapid deep and complete promotions after treatment with Trastuzumab deruxtecan.

Informed consent was obtained from the people who participated in the study.

DISCUSSION

Brain metastases are common in solid tumors, and lung and breast cancers are among the most common cancers with brain metastases. Brain metastases, unlike other solid

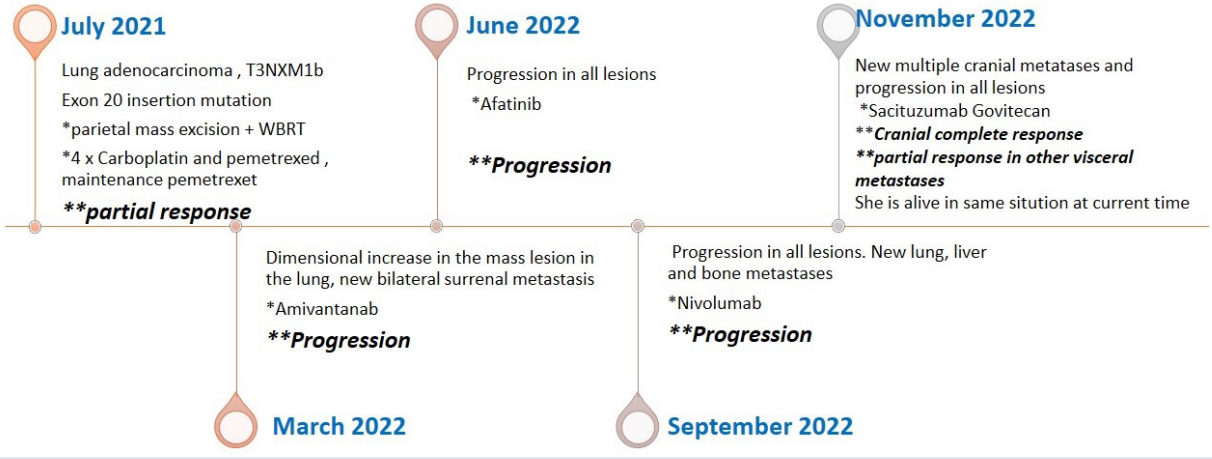

FIGURE 1: Systemic treatments used and the results achieved for the patient who underwent whole brain radiotherapy after surgery.

WBRT: Whole brain radiotherapy

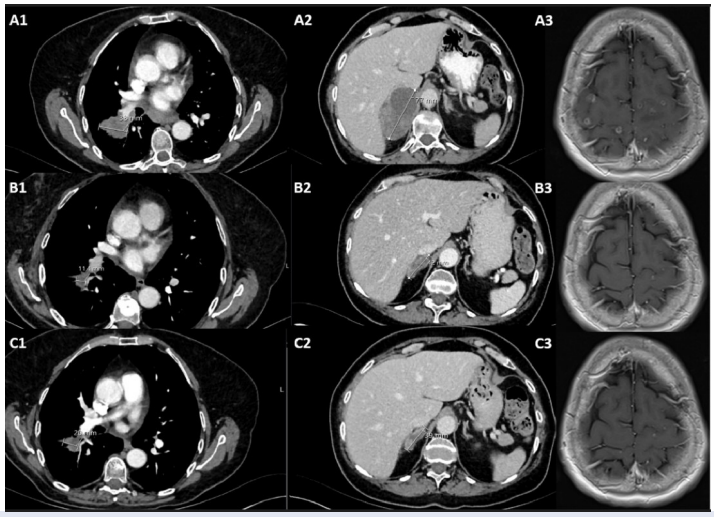

FIGURE 2: Thorax and abdominal computed tomography revealing partial response, and similar findings obtained in the follow-up.

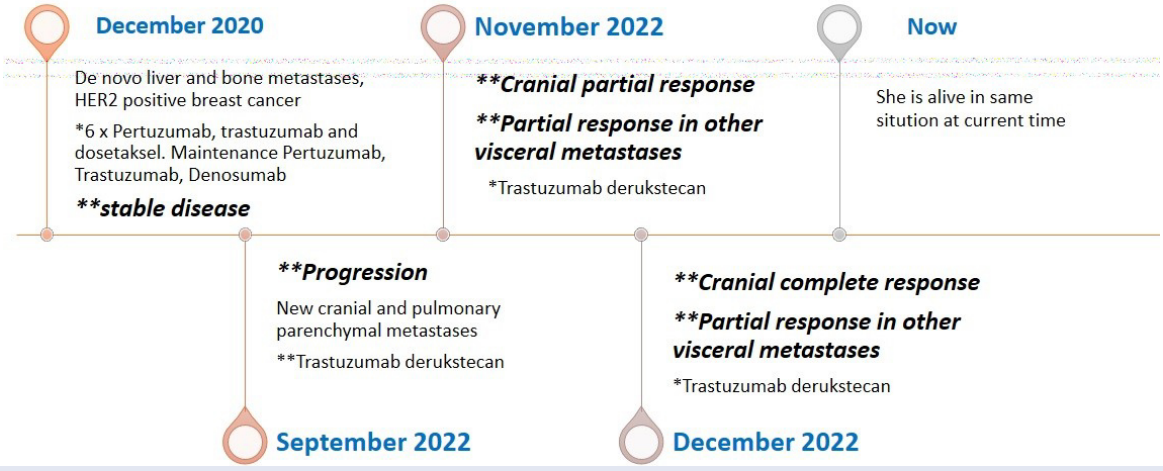

FIGURE 3: Systemic treatments used and the results achieved for the patient.

HER2: Human epidermal growth factor receptor 2

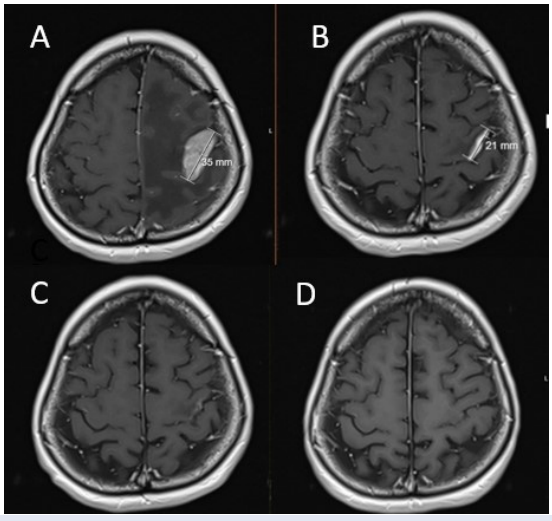

FIGURE 4: Rapid deep and complete promotions after treatment with Trastuzumab deruxtecan.

organ metastases, have a more intratumoral and peritumoral edematous structure. This edematous structure observed in brain tumors and metastases is vasogenic edema that occurs due to impaired blood-brain barrier function and increased vascular permeability.[12] The production of factors that increase tumor vascular permeability, such as VEGF, glutamate, and leukotrienes, and the lack of tight endothelial cell connections within tumor blood vessels are two major factors that cause tumor-related blood-brain barrier disruption and increased permeability.[13] Neovascularization is observed in response to angiogenic factors such as VEGF and fibroblast growth factors (bFGF and FGF2).[14] VEGF is largely responsible for the disruption of blood-brain barrier integrity in gliomas, meningiomas, and metastatic brain tumors, usually through VEGF upregulation.[15] VEGF is released by both tumor cells and stromal cells and is capable of binding to VEGFR1 and VEGFR2, which are receptors located on the

surface of endothelial cells.[16] VEGF stimulates the formation of gaps in the endothelium, resulting in fluid passage across the brain parenchyma, thereby causing vasogenic edema.[17] The newly formed vessels are different from those already present in normal brain tissue, with the former having inadequate expression of the transmembrane proteins occludin and claudin and the intracellular zonula occludin proteins ZO-1, ZO-2, and ZO-3, which are key molecules associated with the abnormalities responsible for the increased permeability of tumor endothelial tight junctions.[18-21] Numerous studies have reported a reduced number of normal astrocytes in brain tumor tissue and the lack of astrocyte-derived factors necessary for the formation of a normal blood-brain barrier as the other causes of defective endothelial tight junctions.[22,23] In addition, high expressions of both aquaporin-1 and aquaporin-4 are reportedly associated with the development of brain edema.[24,25]

Brain metastases are differentiated from other solid organ metastases in terms of their intense edematous structure, which is characterized by the unique factors stated above. Therefore, different systemic treatment options may be used for treating brain metastases with different targets and hemodynamic mechanisms.

Trastuzumab deruxtecan and Sacituzumab govitecan reportedly exhibit efficacy in both breast cancer and NSCLC.[26-29] However, these reports were based on studies that did not evaluate brain metastases separately from systemic diseases. The cases discussed in the present report, however, suggest that efficacy evaluations on brain metastases should be investigated as a separate secondary endpoint in studies conducted on the use of ADCs. In addition, it is recommended that brain metastases, due to their unique pathophysiology and structural features, should be included as a special research topic in the field of antibody-drug design and development.

Brain metastases are among the most detrimental consequences noted in solid tumors, and ADCs may serve as suitable candidates to achieve the solution in this regard.

Ethics

Informed Consent: Informed consent was obtained from the people who participated in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: D.D., İ.B., İ.Ç., Concept: D.D., İ.Ç., Design: İ.B., İ.Ç., Data Collection or Processing: D.D., E.Ö., Analysis or Interpretation: İ.B., İ.Ç., Literature Search: D.D., E.Ö., Writing: D.D., İ.B.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

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