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ORIGINAL ARTICLE

Biomarkers Associated with Clinical Outcome of Advanced Melanoma Patients Treated with Ipilimumab

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Abstract

Ipilimumab was the first immunotherapy approved for metastatic melanoma in decades and is currently registered as a second-line treatment. However, new immunotherapies, in combination with ipilimumab, offer even better clinical outcomes for patients compared with single-agent treatments, at the expense of improved toxicity. The aim of this study was to evaluate the feasibility of ipilimumab outside the clinical trials and to identify survival predictors for treatment benefit. Data were collected on 47 advanced melanoma patients treated with ipilimumab between 2010 and 2015 at a single center. Association of clinical characteristics (including primary tumor characteristics), serum lactate dehydrogenase (LDH), erythrocyte sedimentation rate, absolute eosinophil, lymphocyte, and neutrophil count, neutrophil/lymphocyte and eosinophil/lymphocyte ratio with toxicity and clinical outcome were assessed using univariate and multivariate analysis. Median progression-free survival at a median follow-up of 10 months was 2.7 months and median overall survival was 9.8 months. Objective response was observed in 17% of patients and the disease control rate at week 24 was 40%. The 1- and 2-year survival rates documented were 40 and 28%, respectively. Significant association between high LDH level (>1.5× upper limit of normal) and decreased overall survival was demonstrated in uni- and multivariate analysis (hazard ratio [HR]: 3.554, 95% CI: 1.225–10.306, $p = 0.019$). Neither biomarkers nor clinical outcome were associated with toxicity. Using baseline serum LDH to identify patients most likely to benefit from ipilimumab therapy could serve as a simple and inexpensive biomarker of clinical outcome.

Keywords Melanoma · Ipilimumab · Predictive factors · Lactate dehydrogenase

Introduction

Historically, advanced, unresectable melanoma is a disease with poor prognosis; until recently, patients with metastatic melanoma had a median life expectancy of around 8 months, with limited treatment options that did not impact survival [1, 2]. The approval of targeted drugs and checkpoint inhibitors for the treatment of advanced melanoma has significantly changed patient outcomes. Ipilimumab was the first agent that improved survival of metastatic melanoma patients in a randomized, controlled phase III trial [3]. Long-term survival data of 2985 patients from phase II-III trials showed a median OS of 9.5 months with a plateau at 21% in the survival curve around year 3, with follow-up of up to 10 years [4]. In 2015, FDA approved the combination of nivolumab and ipilimumab for the treatment of BRAF wild-type advanced melanoma based on the 59% response rate compared with 11% response rate for ipilimumab [5]. Moreover, ipilimumab is the first immunotherapy to be approved for the adjuvant treatment of fully resected stage III melanoma [6].

However, in view of the small proportion of patients with objective response and the potential severity of adverse events, identifying predictive biomarkers is an important goal of current research. The selection of patients who are candidates for the treatment is increasingly important as targeted therapies, and other checkpoint inhibitors became also available. A number of potential biomarkers have been investigated. Baseline

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value or therapy-induced change in serum lactate dehydrogenase, C-reactive protein, erythrocyte sedimentation rate, absolute lymphocyte or eosinophil count, and neutrophil-to-lymphocyte ratio (NLR) were found associated with disease outcome [7–22]. Early studies with ipilimumab reported a correlation between favorable clinical outcome and the occurrence of immune-related adverse events [23, 24].

In melanoma, activating mutations of BRAF and NRAS lead to constitutive signaling of the MAPK pathway and promote tumor growth and disease progression [25, 26].The correlation between BRAF and NRAS mutation status and the efficacy of immunotherapy is still a matter of controversy [27, 28].

A major drawback of most of the potential markers is that they become evident only during the course of treatment, thereby making them unsuitable for upfront patient selection. Thus far, no reliable predictive parameter is established in daily clinical routine that can be used for the identification of patients who benefit from ipilimumab. The aim of the present study was to evaluate the efficacy of ipilimumab in clinical practice and the secondary aim was to identify easily accessible biomarkers associated with clinical response and survival. Moreover, we examined whether the occurrence of adverse events after treatment with ipilimumab was associated with clinical outcome.

Patients and Methods

Patients and Treatment

The study was conducted using a research ethics board-approved protocol (Scientific and Ethical Committee of Medical Research Council, Hungary 4758–2/2017/EKU) and was done in accordance with the Declaration of Helsinki. This was a retrospective analysis of a consecutive series of all patients administered with ipilimumab 3 mg/kg for melanoma at the National Institute of Oncology (Budapest) between 2010 and 2015.

Patients were eligible for inclusion if they had a histologically confirmed, unresectable stage III or IV skin, ocular or mucosal melanoma and failed to respond or were intolerant for at least one systemic therapy. An Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1, or 2 was required and an interval of at least 28 days since treatment with chemotherapy, surgery, radiation or immunotherapy was recommended. Exclusion criteria were the presence of an autoimmune disease, HIV, Hepatitis B or C, pregnancy, concomitant malignancies, symptomatic brain metastases.

Patients were treated intravenously with ipilimumab 3 mg/kg every 3 weeks, for a maximum of four doses within the EAP (European Expanded Access Programme) or by financial support of the National Health Insurance Fund. Dose reduction or modification was not allowed, but dose omission or discontinuation was recommended when necessary.

Electronic health records of patients were reviewed and data collected for age, sex, ECOG performance status, site of the primary melanoma, tumor burden, BRAF and NRAS mutation status, metastatic stage according to American Joint Committee on Cancer (AJCC) classification, full blood count and serum factor levels, previous treatments, response on CT scan, adverse events, best response at the time of data cut-off and survival outcomes of progression-free (PFS) and overall survival (OS). All patients signed informed consent that comprised a data privacy clause for data collection and analysis for scientific purposes.

Assessment

Patients underwent a pretreatment evaluation including a physical examination, standard blood testing (liver, renal, thyroid functional test and hemogram) and a radiological evaluation of the disease. During the induction phase, clinical examination, adverse event monitoring and laboratory tests were performed before each drug infusion. CT scans of the brain, chest, abdomen and pelvis were carried out at 12, 16 and 24 weeks after the first ipilimumab infusion.

Response was classified as immune-related complete response (irCR), partial response (irPR), stable disease (irSD) and progressive disease (irPD) according to Immune-related Response Criteria – irRC [29]. Durable disease control (DC) was defined as SD at least 12 weeks from the first dose, CR or PR. Clinical benefit was represented by immune-related best overall response rate (irBORR: irCR or irPR) and disease control rate (irDCR: percentage of patients achieving irCR, irPR or irSD).

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.0.

Candidate biomarkers, comprising LDH, erythrocyte sedimentation rate (ESR), absolute lymphocyte, neutrophil and eosinophil counts (ALC, ANC, AEC) were evaluated in peripheral blood or serum samples collected within 10 days before the first ipilimumab dose. Full blood count analysis was performed with an automatic cell counter using the coulter principle (normal ranges for full blood count variables were neutrophils 1.9–7 G/L, lymphocytes 4–11 G/L, eosinophils 0.04–0.4 G/L, LDH range 226–451 U/L and ESR range 2–20 mm/s). Neutrophil/lymphocyte ratio (NLR) and eosinophil/lymphocyte ratio (ELR) were also determined.

The grading of tumor-infiltrating lymphocytes (TILs) has been assessed in a semiquantitative way on HE-stained slides of the primary melanoma according to the protocol of the College of American Pathologists, 2015 (based on AJCC/UICC TNM, 7th edition). We qualified TILs as absent if there was no lymphocytic infiltration or the lymphocytes

were present but did not infiltrate the vertical growth phase. Mutations were tested using polymerase chain reactions (PCR) covering BRAF exon 15 (codon 600), NRAS exon 2 (codons 12, 13) and NRAS exon 3 (codon 61), preferentially in metastases. If no such samples were available, analyses were performed on primaries.

Statistical Analysis

Descriptive statistics were used to present patient's characteristics, safety and efficacy of treatment. Mann-Whitney U-test and Fisher's exact test were used to evaluate the association of baseline variables with clinical response. Progression-free survival (PFS), defined as the time from first treatment to immune-related disease progression or death or last follow-up, and overall survival (OS), defined as the time from first treatment to death or last follow-up were estimated with Kaplan-Meier test. Median follow-up time was estimated by the reverse Kaplan-Meier method.

Prognostic models for PFS and OS using baseline blood cell counts and serum markers were derived using binary partitioning algorithm. The cut-off values for these parameters were defined as AEC of 0.1 G/L, ALC of 1 G/L, ANC of 4 G/L, ELR of 0.1 and NLR of 4. ESR and LDH were categorized using a threshold of ULN and 1.5 × ULN, respectively. The log-rank test was used for univariate analysis to assess the association of patient characteristics and blood parameters with PFS and OS.

Cox proportional hazard regression model was applied to determine the impact of confirmed single factors. Results were presented as hazard ratios (HRs) with 95% confidence intervals (95% CIs). Patients with missing data in variables analyzed in the given model were excluded.

All analyses were carried out using the Statistica software version 12.5 (Statsoft, Tulsa, OK). Throughout the analysis, p values <0.05 were considered statistically significant.

Results

Patient Characteristics

From December 2010 to July 2015, 47 patients received ipilimumab, 34 patients within the Expanded Access Programme and 13 patients after licensing. Patient and treatment characteristics are presented in Table 1. Most patients ($n = 35$, 75%) completed the four cycles of ipilimumab therapy. Two patients (4%) interrupted the treatment before completion because of toxicity and 10 (21%) developed rapid worsening of performance status, requiring interruption of therapy.

Following ipilimumab, 23 (49%) patients received at least one line of systemic treatment: 6 of them (13%) received BRAF inhibitors, 6 (13%) anti-PD-1 drugs and 18 (38%)

Table 1 Patient characteristics

Characteristics	Values
Total number of patients	47
Age, years – median (range)	57 (26–83)
Male, n (%)	27 (57)
T stage of primary, *n* (%)	
T1-T2	15 (32)
T3-T4	25 (53)
Unknown	7 (15)
Primary site, *n* (%)	
Cutaneous	44 (94)
Uveal	2 (4)
Mucosal	1 (2)
TIL of primary, *n* (%)	
Present	19 (40)
Absent	18 (38)
Unknown	10 (21)
ECOG performance status, *n* (%)	
0	35 (74)
1	12 (26)
2	0
AJCC stage, *n* (%)	
Stage III (unresectable)	6 (13)
Stage IV M1a	6 (13)
Stage IV M1b	10 (21)
Stage IV M1c	25 (53)
Brain metastasis at baseline, *n* (%)	
No	41 (87)
Yes	6 (13)
Number of previous treatments, *n* (%)	
1	19 (40)
≥ 2	28 (60)
LDH level at baseline, *n* (%)	
≤ 1.5 × ULN	35 (74)
> 1.5 × ULN	11 (23)
Unknown	1 (2)
Number of metastatic organs, *n* (%)	
< 3	34 (72)
≥ 3	13 (28)
Cycles of ipilimumab, *n* (%)	
1	4 (8)
2	2 (4)
3	6 (13)
4	35 (75)
Mutation status, *n* (%)	
BRAF+NRAS-	18 (38)
BRAF-NRAS+	8 (17)
BRAF-NRAS-	13 (28)
Unknown	8 (17)

patients were administered with cytotoxic therapy. Three (6%) patients received ipilimumab reinduction.

Significant association was found between the number of metastatic organs (NMO) and LDH level. Median LDH level of patients with more than two metastatic organs was 799 U/L, while it was 455 U/L in patients who had only two or less affected sites ($p = 0.0221$). Patients with multiorgan disease (NMO ≥ 3) had an elevated LDH level in 6 of 12 cases (50%) while it was only 15% (5/34) among patients with smaller tumor burden ($p = 0.0223$). LDH values were under 1.5xULN in 27 of 31 (87%) NRAS wild type patients compared to 4 of 8 (50%) NRAS mutant cases ($p = 0.0401$).

Toxicity

Of the 47 evaluated patients, 19 (40%) experienced immune-related adverse events, including 6 (13%) grade 3–5 events (Table 2). The most common irAEs were grade 1–2 dermatologic reactions ($n = 17$, 36%) and gastrointestinal toxicities ($n = 6$, 13%, including two grade 3–5 events). One patient died from colitis, considered possibly treatment-related (2%). Two patients experienced grade 3–4 nephritis and one patient developed grade 3 orbital inflammation. Other serious irAEs included adrenal insufficiency in two patients with evidence of hypophysitis on magnetic resonance imaging. Treatment was discontinued because of grade 3 nephritis or grade 4 colitis in two cases.

Clinical Response

Results for clinical response are summarized in Table 3. Five patients (6%) were not evaluable for response due to rapid deterioration and death before week 12. The best overall response rate (irBORR) was 17% (12% irCRs and 5% irPRs). Ten patients (24%) experienced immune-related SD as their best response, whereas the remaining 25 patients (60%) had immune-related PD. Disease-control rate at week 24 was 40%.

Investigating serum and blood parameters as well as clinicopathologic characteristics for possible associations with treatment response, we found that baseline AEC and ELR were higher in patients with progressive disease when compared with non-PD patients at week 12. The difference remained significant at week 16 but it disappeared at week 24. Median ESR was higher in PD patients when compared with non-PD patients at week 24, while ALC, ANC, NLR, and LDH were comparable in PD patients and in patients with DC at all time points (Table 4). None of the clinical characteristics studied (as age, sex, performance status, site of the primary melanoma, tumor burden, metastatic stage, previous treatments, presence of brain metastasis) correlated with clinical response. Durable disease control rates (at 24 weeks) were comparable among patients with BRAF mutant or wild type tumors ($p = 0.5742$), and among patients with NRAS mutant or wild type tumors ($p = 0.5445$). None of the blood count parameters or clinical characteristics was significantly associated with irAEs (data not shown).

Table 2 Immune-related adverse events

Toxicity	All grades, *n* (%)	Grade 3–5, *n* (%)
Any	19 (40)	6 (13)
Dermatologic		
Pruritus	1 (2)	0
Rash	15 (32)	0
Vitiligo	1 (2)	0
Gastrointestinal		
Diarrhoea	4 (9)	0
Colitis	2 (4)	2 (4)
Endocrine		
Hypopituitarism	2 (4)	2 (4)
Hepatic	6 (13)	0
Other		
Nephritis	2 (4)	2 (4)
Uveitis	1 (2)	1 (2)

Table 3 Tumor response and survival after ipilimumab therapy

Best overall response (42 patients)	
Complete response	5 (12%)
Partial response	2 (5%)
Stable disease	10 (24%)
Progression	25 (60%)
Best overall response rate	17%
Disease control rate	40%
PFS, months – median (95% CI)	2.7 (0.1–5.4)
OS, months – median (95% CI)	9.8 (4.7–14.9)
6-month OS	70%
12-month OS	40%
24-month OS	28%

Survival

As of December 2016, median PFS at a median follow-up of 10 months (range: 1–61) was 2.7 months (95% CI: 0.1–5.4). Univariate analysis of pretreatment patient characteristics revealed that LDH > 1.5 × ULN (HR: 2.297, 95% CI: 1.105–4.777, $p = 0.0323$), ESR > 1 × ULN (HR: 1.756, 95% CI: 0.926–3.329, $p = 0.0340$) and AEC > 0.1 G/L (HR: 1.909, 95% CI: 1.010–3.609, $p = 0.0238$), but none of the other clinicopathologic parameters examined, were significantly correlated with diminished PFS. Disease progression rates were

Table 4 Comparison of baseline continuous variables in patients with disease control (DC) vs. patients with progressive disease (PD)

	Week 12			Week 16			Week 24		
	DC	PD	*p* value	DC	PD	*p* value	DC	PD	*p* value
LDH (G/L)	479	514	0.8831	463	535	0.4203	354	530	0.1677
ESR (mm/h)	30	45	0.1079	29	45	0.0532	18	46	**0.0059***
ANC (G/L)	4.2	4.6	0.6981	4.3	4.4	1.0000	3.7	4.7	0.2455
AEC (G/L)	0.08	0.16	**0.0042***	0.09	0.16	**0.0299***	0.08	0.12	0.1180
ALC (G/L)	1.2	1.16	0.9042	1.18	1.16	0.9214	1.17	1.25	0.5574
NLR	2.7	3.3	0.7789	2.8	4.0	0.6213	3.0	5.8	0.5105
ELR	0.08	0.18	**0.0066***	0.09	0.19	**0.0257***	0.09	0.15	0.1677

*Bold values indicate statistical significance ($p<0.05$)

found independent of BRAF ($p = 0.3952$) and NRAS ($p = 0.5768$) mutation status. In a multivariate analysis including LDH, ESR and AEC, no variable remained significantly associated with disease progression.

The median OS observed from the first cycle of ipilimumab was 9.8 months (95% CI: 4.7–14.9), with a 1-and 2-year survival rate of 40 and 28%, respectively (Table 3). Forty deaths were observed during follow-up (39 melanoma-related, one was due to the therapy).

Univariate analysis showed that factors significantly associated with diminished OS were LDH > 1.5 × ULN, ESR > 1 × ULN, NLR ≥ 4, AEC > 0.1 G/L, ELR > 0.1, performance status >0

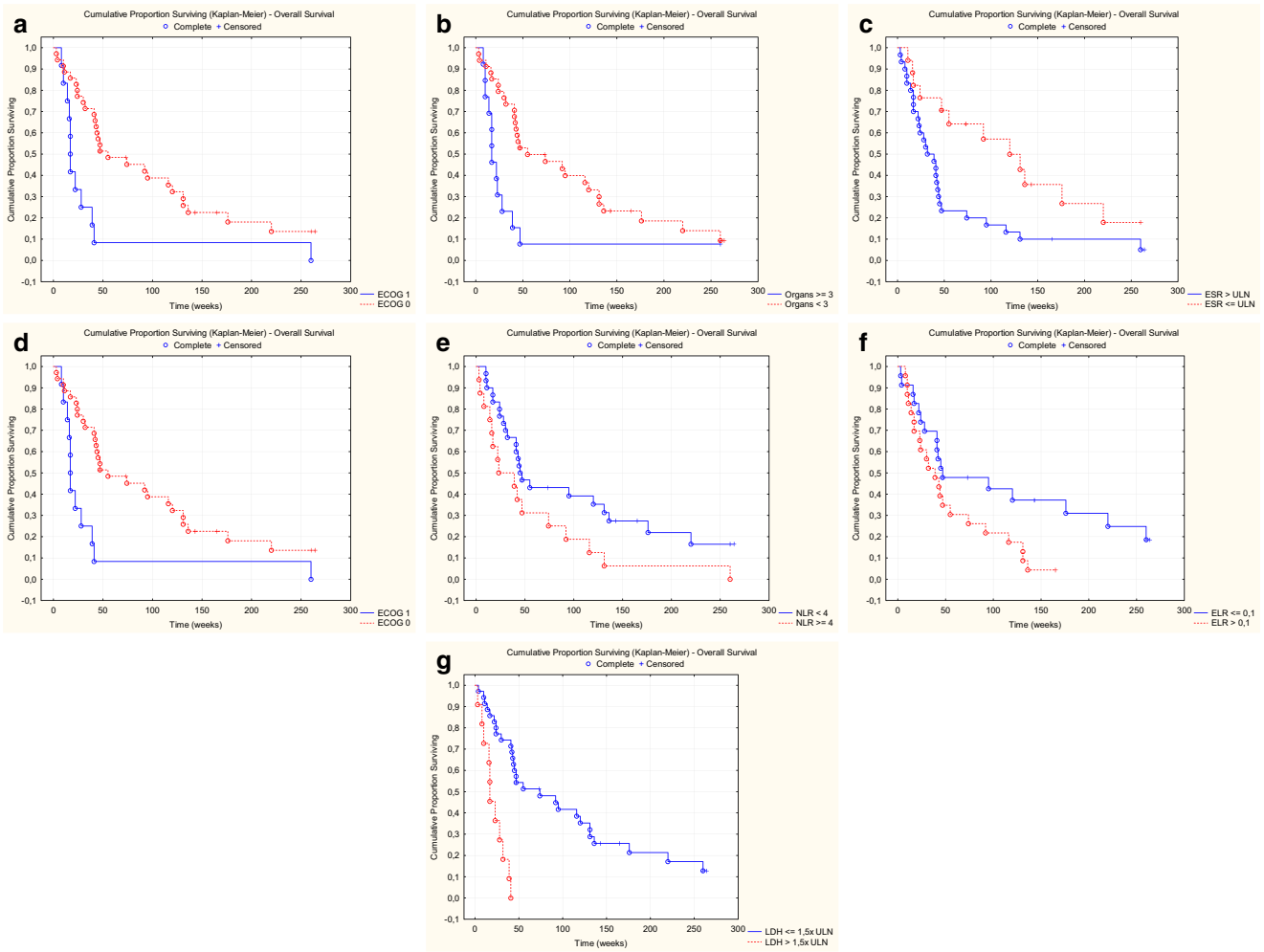

Fig. 1 Kaplan-Meier survival curves of patients categorized according to ECOG performance status (**a**), number of metastatic organs (**b**), ESR (**c**), AEC (**d**), NLR (**e**), ELR (**f**), or LDH (**g**)

Table 5 Univariate analysis of baseline characteristics and overall survival (log-rank test)

	Hazard ratio	95% CI	*p* value
ECOG performance status >0	2.898	1.427–5.886	**0.0153***
Number of metastatic organs ≥3	2.555	1.265–5.159	**0.0300***
ESR > 1 × ULN	2.367	1.884–4.716	**0.0084***
AEC > 0.1 G/L	2.304	1.142–4.648	**0.0193***
NLR ≥ 4	1.970	1.035–3.750	**0.0475***
ELR > 0.1	6.105	1.302–25.628	**0.0401***
LDH > 1.5 × ULN	6.565	2.695–15.996	**0.0029***

*Bold values indicate statistical significance ($p<0.05$)

and multi-organ disease (Fig. 1, Table 5). The other clinicopathologic parameters studied (age, primary tumor T-classification and presence of TILs, metastatic stage, number of previous treatments, presence of irAES), ALC or ANC were not significantly associated with OS, however, AJCC stage had a strong trend toward significance ($p = 0.078$, data not shown). In this study, overall survival was also independent of BRAF ($p = 0.6355$) and NRAS ($p = 0.6659$) mutation status.

In multivariate analysis, LDH level > 1.5 × ULN was significantly and independently associated with shorter overall survival, adjusting for the most relevant significant prognostic factors (Table 6). Patients with a baseline LDH ≤ 1.5 × ULN had a 3.5-fold reduced risk of death when compared with those with elevated LDH level. Median OS was 17 months for patients with LDH ≤ 1.5 × ULN and only 4 months for patients with LDH > 1.5 × ULN. The one- and two-year survival rates were 54 and 42% for the LDH-low patients compared with 0% in the LDH-high group.

Discussion

The clinical response to ipilimumab demonstrated in 47 heavily pretreated patients was similar to those observed in previous trials. Our objective response rate of 17% is comparable to the objective RRs of 10 to 26% that have been reported in other studies. These trials also reported durable disease controls of 28.5 to 41% similar to our DC of 40% [3, 7, 11–13, 28]. In 2 patients a discrepant tumor response was observed when using irRC as opposed to Recist 1.1 (changing a best response of 2 cases with progressive disease into PR and CR).

Table 6 Multivariate analysis of the association of baseline characteristics and overall survival

Parameters	Hazard ratio	95% CI	*p* value
ECOG performance status	1.653	0.644–4.240	0.2954
LDH	3.554	1.225–10.306	**0.0190***
AEC	1.507	0.578–3.924	0.4000
ESR	1.544	0.609–3.912	0.3590

*Bold values indicate statistical significance ($p<0.05$)

The survival outcomes are, interestingly, similar to those of the ipilimumab registration trial [3] despite the wider eligibility criteria in EAP compared with the randomized clinical trial (6% of patients had extracutaneous melanoma, 28% of them had three or more metastatic sites, and 13% had metastasis of the brain).

We report a median PFS of 2.7 months, and a median OS of 9.8 months, again highly comparable with the previously reported 2.6 to 3.7 months and 5 to 10.1 months observed in prospective and retrospective studies using the same dosing regimen of ipilimumab in pretreated melanoma patients. We observed 40 and 28% 1- and 2-year survival rates, respectively, which is in line with the previously reported 34.8 to 46% and 22 to 28.8% survival rates [3, 7, 8, 10–13, 16, 20, 28].

We observed a plateau in the survival curve at 2.7 years, consistent with previous studies of ipilimumab. In all analyses, the survival curves consistently begin to plateau around 3 years with follow up to 10 years in a small proportion of patients [4].

The irAEs reported were consistent with the results of earlier studies involving ipilimumab at a dose of 3 mg/kg [3, 10–13, 16, 28]. IrAEs were generally mild and manageable, the most common irAE was dermatitis. However, there was one treatment-related death due to severe colitis resulting in bowel perforation. Two patients suffered from irreversible hypopituitarism with isolated adrenocortical insufficiency. The occurrence of rare serious irAEs was observed in 3 patients: one with grade 3 orbital inflammation and two with grade 3 nephritis. Early clinical studies reported a correlation between the occurrence of irAEs and favorable clinical outcome [7, 23, 24]. In contrast to these findings, occurrence of AEs was not found to correlate with either survival or best clinical response in our study.

Evaluating the potential biomarkers of clinical outcome, we focused exclusively on the impact of clinical parameters and factors available in the routine laboratory setting. It was recently suggested that performance status and the number of organs involved were independent prognostic factors for OS in patients with metastatic melanoma treated with ipilimumab [15]. In our study, clinical response appeared independent of baseline patient's characteristics, however, worse performance status and high number of organs involved had a negative influence on survival in univariate analysis.

AJCC stage approached the borderline of significance in univariate analysis, although a strong impact has been demonstrated in other studies [30, 31]. Similarly, neither age, sex, primary tumor characteristics nor previous treatments had independent prognostic impact on survival in our analysis.

Studies on the prognostic relevance of mutation status have revealed discordant results. A recent retrospective study identified NRAS as an independent favorable factor for OS, compared to BRAF mutated and wild genotype [32]. Others have

found that NRAS mutational status predicted shorter survival [33, 34] or no difference in survival, regardless BRAF and NRAS mutation status in metastatic disease [35, 36]. We observed a significant association between NRAS+ mutational status and high LDH levels, however, efficacy of ipilimumab was independent of BRAF and NRAS genotype.

In the last decade it has become evident that cancer-related inflammation may influence outcome in a variety of cancer types [37–39]. ESR is a marker of inflammation; we found it significantly higher in patients who had PD at week 24 than in those who had DC at this time and elevated ESR had a negative influence on PFS and OS in univariate analysis.

Systemic inflammation is associated with changes in the number and function of immune cells and soluble factors. It has been hypothesized that tumor microenvironment, which is believed to play a role in determining the response to immunotherapy, could be influenced by neutrophils. High neutrophil count indicates systemic inflammation that can promote tumor growth [40, 41], and baseline neutrophil count was found to be an independent negative prognostic factor [19, 42].

Association between baseline ALC and OS of patients treated with ipilimumab was observed in recent studies [12, 14], likewise, correlation was observed between the rate of increase in ALC and survival [7, 10, 11, 13]. Furthermore, tumor-infiltrating lymphocytes are associated with good prognosis in a number of tumor types, including melanoma [43, 44]. Baseline ANC and ALC were incorporated in the NLR, and high baseline NLR was associated with poor survival in many cancers [38, 39]. Connection of baseline NLR and OS in melanoma patients treated with ipilimumab was reported in several recent studies [9, 16, 20, 22]. We observed that pretherapy low NLR (<4) was significantly associated with improved OS but not with PFS. However, low NLR was not of prognostic value in our multivariate analysis, although a strong impact has been shown in other studies.

Recent studies supported a potential role for eosinophils as effector cells in tumor rejection. [45, 46] Association between high baseline AEC and improved OS with ipilimumab therapy was reported in one study [21]. In others, however, no such correlation was seen, while therapy-induced increase in eosinophil count was found connected to improved survival [10, 17]. In the current study, a negative correlation was observed between median baseline AEC or ELR and early clinical response. In univariate analysis, elevated baseline serum AEC and ELR had a negative influence on survival.

High disease load and cell turnover, resulting in elevated serum levels of LDH, is a well-known negative prognosticator in stage IV melanoma (2009 AJCC melanoma staging). However, in the registrational trial [3] survival was found to be independent of LDH. Recent studies suggest that LDH is a predictive marker of treatment outcome [8, 10, 12, 15, 18, 20]. In the present study, LDH was the only biomarker that was significantly associated with OS both in univariate and multivariate analysis. The confirmation of the prognostic value of LDH is consistent with the well-established importance of this marker for melanoma.

In conclusion, tumor response rate, safety and survival with ipilimumab at the dose level of 3 mg/kg were consistent with previous studies of ipilimumab in patients with pretreated advanced melanoma and long-term benefit of treatment was unlikely for patients with baseline serum LDH greater than 1.5× upper limit of normal.

After all, in routine clinical practice considering performance status, number of affected organs, erythrocyte sedimentation rate, eosinophil count, neutrophil to lymphocyte and eosinophil to lymphocyte ratio beside LDH to identify patients most likely to benefit from ipilimumab therapy could serve as inexpensive biomarkers of clinical outcome.

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Compliance with Ethical Standards

Conflicts of Interest Tímea Balatoni has received speaker honoraria and financial support for attending symposia from Bristol-Myers Squibb, Merck Sharp & Dohme (MSD), Novartis, and Roche. Gabriella Liszkay is on the advisory board and has received honoraria for speaking at conferences as well as financial support for educational programs from Bristol-Myers Squibb, GlaxoSmithKline, MSD, Novartis, and Roche. Gitta Pánczél and Kata Czirbesz has received speaker honoraria from Bristol-Myers Squibb, MSD, Novartis, and Roche. All other authors declare that they have no conflict of interest.

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