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ORIGINAL ARTICLE

Effect of glucocorticoids for the management of immune-related adverse events on outcome in melanoma patients treated with immunotherapy—a retrospective and biomarker study

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Background: Immune-related adverse events (IRAEs) during therapy with immune checkpoint inhibitors (ICIs) are common, and their management sometimes requires glucocorticoids (GCs). Predictors for development of IRAEs and data about the impact of GCs on clinical outcome are missing. We evaluated the impact of GCs to treat IRAEs on clinical outcome, and plasmatic inflammatory proteins as predictors for IRAEs.

Patients and methods: Patients with melanoma ($n = 98$) treated with ICIs at Karolinska University Hospital were included. Clinical information and data regarding prescription of systemic GCs were collected. Baseline plasma samples ($n = 57$) were analyzed for expression of 92 inflammatory proteins.

Results: Forty-four patients developed at least one IRAE requiring systemic GCs and the most common was hypocortisolemia ($n = 11$). A median overall survival of 72.8 months for patients developing IRAEs requiring GCs, 17.7 months for those who did not, and 1.4 months for individuals receiving GCs at baseline was observed in Kaplan—Meier curves ($P = 0.001$). In immortal time bias adjusted analysis, patients receiving steroids to treat IRAE survived slightly longer, even though this time trend was not statistically significant. The median overall survival was 29 months for those treated with GCs within 60 days after ICIs start and was not reached for patients receiving GCs later. The number of ICI cycles was higher in subjects receiving GCs after 60 days ($P = 0.0053$). Hypocortisolemia occurred mainly in males (10/11) and correlated with favorable outcome. Male patients with hypocortisolemia had lower expression of interleukin 8, transforming growth factor-α, and fibroblast growth factor 5 and higher expression of Delta/Notch-like epidermal growth factor-related receptor.

Conclusions: GCs may be used to treat IRAEs without major concern. GCs early during ICIs may, however, impact clinical outcome negatively. The prognostic value of hypocortisolemia and inflammation proteins as biomarkers should be further investigated.

Key words: melanoma, immunotherapy, toxicity, glucocorticoids, inflammation, proteins

INTRODUCTION

Immune checkpoint inhibitors (ICIs) block inhibitory immune signals such as programmed cell death protein 1 inhibitor (anti-PD-1) and cytotoxic T-lymphocyte-associated antigen 4 inhibitor (anti-CTLA-4) and are well established therapy options for patients with advanced cutaneous malignant melanoma (CMM).[1-3] They activate the immune response against tumor cells and have dramatically improved patients' outcome during the last decade.[1-3] These drugs can be used as single agents but have proven to be more effective when given in combination. The 6.5-year overall survival (OS) for patients with metastatic CMM treated with nivolumab (anti-PD-1) in combination with ipilimumab (anti-CTLA-4) is 49% compared with 42% and 23% when nivolumab and ipilimumab were given as single drugs, respectively.[3]

Even if approximately half of the patients have a *BRAF*-mutated melanoma[4] where tumor growth can be rapidly suppressed by treatment with mitogen-activated protein kinase (MAPK) inhibitors (BRAF and MEK inhibitors),[5-7] the treatment of first choice for most patients with metastatic CMM is ICIs.[8] ICIs are also being used in the adjuvant and neoadjuvant setting in CMM improving disease-free survival

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in individuals with operable locoregional more advanced disease.[9-11]

By activating the immune system, ICIs can cause immune-related inflammation in any organ in the body as a side-effect.[12] In the CheckMate-067 study, which compared nivolumab or ipilimumab alone with the combination of both drugs in advanced CMM, the authors observed that immune-related adverse events (IRAEs) of any grade occurred in 82%, 86%, and 95%, respectively. The incidence of high-grade IRAEs was also higher in the combination arm (55.0%) compared with the nivolumab arm (16.3%) and the ipilimumab arm (27.3%).[13] IRAEs often require the prescription of immune suppressors and the most commonly used are glucocorticoids (GCs).[12] In the CheckMate-067 study, immune suppressive drugs to treat IRAEs were indicated for 47% of subjects in the nivolumab arm, for 83% of those in the combination arm, and for 56% of patients in the ipilimumab arm.[13]

The assumption that the development of IRAEs indicates activation of the immune system against tumor cells and that IRAEs may be a surrogate marker for ICI efficacy has been studied in many tumor types with contradictory results.[14,15] A meta-analysis of 30 studies including 4971 subjects with different cancers found that low-grade IRAEs were associated with favorable OS whereas high-grade IRAEs were not.[14] A speculative explanation of this finding could be that grade 3 or 4 IRAEs are more likely to be treated with higher doses of GCs and that these patients may have had their ICI interrupted prematurely.

GCs inhibit inflammation and are immunosuppressive by regulating the expression of some anti-inflammatory and inflammatory mediators.[16] Nonetheless, data about the impact on ICIs efficacy of systemic GCs prescribed to manage IRAEs in CMM has been contradictory and further studies are needed.[17-21]

In summary, the impact of IRAEs requiring systemic GCs (+GCIRAE) on OS of CMM patients treated with ICIs is still poorly understood, and clinical factors and biomarkers to predict the risk of developing IRAEs are unmet needs. They would contribute to more individualized treatment choices and prophylactic strategies. We have therefore carried out a retrospective study of 98 patients treated with ICIs for metastatic CMM to evaluate whether +GCIRAE and its onset timing impact the clinical outcome for these patients. We also investigated if inflammation-related proteins in pretreatment plasma from 57 of the patients could predict the development of +GCIRAE by utilizing the Olink® inflammation protein platform.

MATERIAL AND METHODS

Study design and patient cohort

This is a retrospective study including 98 patients with metastatic CMM treated with ICIs at Karolinska University Hospital, in Stockholm, between 2012 and 2020. We collected clinical information from patients' medical records such as age, sex, metastatic classification (M1-class) according to the 8th American Joint Committee on Cancer (AJCC) staging edition,[22] baseline lactate dehydrogenase (LDH) level, type, and line of ICI (Table 1). Data about the use of systemic GCs (any type and dose) to treat IRAEs, start date of GCs and information about the use of GCs at a dose corresponding to at least 10 mg prednisolone/day within 30 days before the start of ICI treatment were also retrospectively collected (Table 1). If a patient received GCs to treat an IRAE, the type of IRAE was assembled (Supplementary Table S1, available at https://doi.org/10.1016/j.iotech.2024.100713). The GCs of clinician's choice to treat IRAEs varied and different agents were prescribed, i.e. hydrocortisone, methylprednisolone, betamethasone, and prednisolone. We converted all GCs into prednisolone dose equivalents to compare the GCs start dose in the two +GCIRAE subgroups. Infusion-related reactions were not considered IRAEs and were not included in the analysis.

Six patients were treated within the clinical trial NCT02752074 and were followed according to the study protocol. The other patients were followed according to our clinical routine which means that therapy response evaluation is based on joint decision of clinical/radiological

Table 1. Summary of patients' baseline clinical characteristics.

	−GCIRAE (n = 54)	+GCIRAE (n = 44)	*P* value
Age, years			
Median (range)	71 (24-84)	66 (31-84)	0.131
Gender, *n* (%)			
Female	19 (35.2)	15 (34.1)	1.000
Male	35 (64.8)	29 (65.9)	
M Stage, *n* (%)			
M1a or M1b	28 (51.9)	25 (56.8)	0.686
M1c or M1d	26 (48.1)	19 (43.2)	
LDH[a] (%)			
Normal	22 (40.7)	20 (45.5)	0.836
Elevated	30 (55.6)	23 (52.3)	
Not available	2 (3.7)	1 (2.3)	
Therapy[b,c] *n* (%)			
Nivolumab	40 (74.1)	23 (52.3)	0.135
Pembrolizumab	12 (22.2)	13 (29.5)	Single versus combination
Pembrolizumab + epacadostat	—	2 (4.5)	
Nivolumab + ipilimumab	2 (3.7)	6 (13.6)	
Previous lines of treatment, *n* (%)			1.000
0	46 (85.2)	37 (84.1)	
≥1	8 (14.8)	7 (15.9)	
Baseline GC[d], *n* (%)			
Yes	6 (11.1)	0 (0)	
No	48 (88.9)	44 (100)	
Baseline antibiotics[e], *n* (%)			
Yes	12 (22.2)	11 (25)	0.813
No	42 (77.8)	33 (75)	

The patients are divided into those who received glucocorticoids (GC) due to immune-related adverse events (+GCIRAE) and those who did not (−GCIRAE).
LDH, lactate dehydrogenase.
[a]Pretreatment LDH levels were not available from two patients due to hemolysis.
[b]Six patients were treated within the clinical trial NCT02752074. Two of them received pembrolizumab plus epacadostat.
[c]Pembrolizumab + epacadostat was considered as single therapy since epacadostat had no effect.
[d]Six patients received glucocorticoids within 30 days before ICI therapy.
[e]Twenty-three patients received antibiotics within 30 days before ICI therapy.

investigations evaluated by a team including oncologists and radiologists. Clinical visits and radiological evaluations are carried out every 3 months.

Our study was carried out following Good Clinical Practice/the Declaration of Helsinki and ethical approval was obtained from the Stockholm Regional Ethics Committee, Sweden. Informed consent was obtained from all participants in this study.

Plasma samples

Peripheral blood samples (∼10 ml) were collected in EDTA vacutainer tubes before treatment start with ICI therapy and centrifuged at 1500 × *g* for 10 min at room temperature to separate plasma. Plasma was collected and centrifuged again at 2400 × *g* for 15 min at room temperature. Plasma samples were stored in aliquots at −80 °C until analyses.

Multiplex proteomics analysis

We used the Proximity Extension Assay (PEA) technology to assess 92 low-abundant inflammation proteins in plasma samples (https://olink.com/). Baseline plasma samples from 57 of the 98 patients were analyzed by Olink Proteomics in Uppsala, Sweden, utilizing the multiplex proteomics Olink inflammation protein panel, who also carried out data quantification, normalization, and standardization. Normalized data was thereafter analyzed by the software Qlucore Omics Explorer version 3.9. Proteins with >50% of the samples below Olink's predetermined limit of detection (LOD) were discarded. Multiple testing correction was carried out using Benjamini−Hochberg and Holm−Bonferroni. Receiver operating characteristic (ROC) curve analyses were carried out to evaluate the predictive capacity of a subset of potential biomarkers in discriminating between patients dead or alive at 2 years after ICI therapy. The predictive performance is defined to be the resulting area under the curve (AUC).

Statistical analyses

The progression-free survival (PFS) time was calculated from the day of treatment start until the date of progress or death, whatever came first or last follow-up. The OS time was calculated from the day of treatment start until the date of death or last follow-up.

All analyses were done using the statistical software GraphPad Prism version 9.5.1 and STATA version 18. Differences in bivariate associations between clinical variables were tested using Fisher's exact test. For continuous data *P* values were calculated using the Mann−Whitney test. We used the Kaplan−Meier method to estimate PFS and OS. Estimates of PFS and OS curves were compared using the log-rank test and described together with 95% confidence intervals (95% CI) and hazard ratio (HR). Significance is expressed as *P* values <0.05.

Our primary aim was to evaluate how prescription of GCs to treat IRAE affects PFS and OS. A complicating factor is that a patient who lives longer may have an increased risk of experiencing an IRAE needing GC treatment. This phenomenon is called immortal time bias. To adjust for the immortal time bias, Cox regression models were fit with GCIRAE as a longitudinal covariate applying R version 4.3.1 and using the survival and rstpm2 packages.

A longitudinal covariate may change values during the observational period of a patient. All patients had GCIRAE equal to 0 at the start of observation, and for patients experiencing an IRAE needing GC treatment, GCIRAE was set to 1 from that time point onwards.

The final multivariate analyses for PFS and OS were also estimated as a flexible parametric model, to see if differences in survival between −GCIRAE and +GCIRAE changed over time. The flexible parametric model allows the two hazard functions to vary in relation to each other, as opposed to the Cox model in which the hazard functions are proportional between groups. Our models were fit with a baseline natural splines smoother of the log of the time from ICI start with three degrees of freedom, and an interaction between GCIRAE and a natural spline smoother of log(time) with two degrees of freedom.

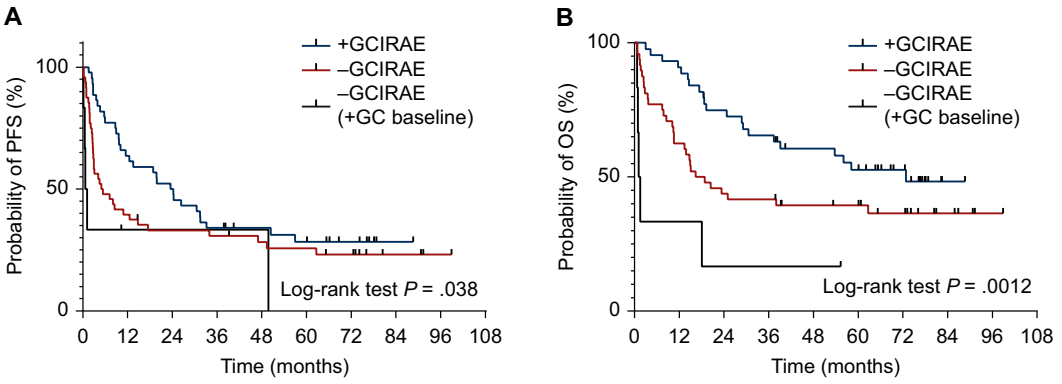
RESULTS

Baseline clinical characteristics did not predict risk for developing +GCIRAE

Clinical characteristics are summarized in Table 1. Most patients were male (65%), and the median age was 70 years old. Forty-six percent of the patients had stage M1c/d and 56% had elevated baseline LDH levels. Most patients (92%) received anti-PD-1 single treatment and ICIs were mostly (85%) given as first-line therapy (Table 1).

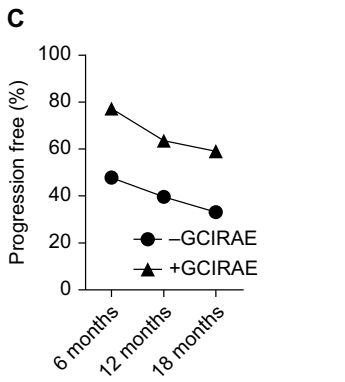
The total number of cases developing at least one +GCIRAE was 44, whereas 54 did not develop IRAEs requiring systemic GCs (−GCIRAE). A total of 6 out of the 54 patients in the −GCIRAE group had received GCs at a dose corresponding to at least 10 mg/day prednisolone at baseline (Table 1). Four of them were on cortisone at baseline to manage cancer-related symptoms (brain metastases and nerve compression) whereas two subjects were on steroids for management of underlying conditions. Three out of these six patients were being treated with ICIs in later lines (second and third)

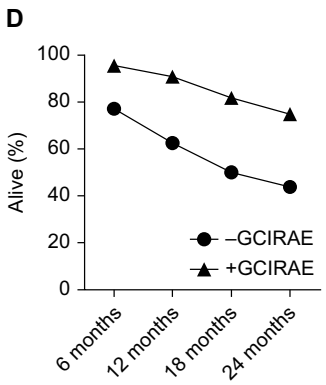
We investigated whether age, sex, M1 stage, LDH levels, single versus combination ICIs, previous lines of treatments, and the use of antibiotics within 30 days before the start of ICIs could affect the risk for developing +GCIRAE. None of these factors differed between the −GCIRAE and +GCIRAE groups (Table 1). Immune suppressive drugs to treat IRAEs are more commonly indicated for combination treatment than single treatment.[13] Single versus combination ICIs, however, did not reach significance regarding the development of +GCIRAE ($P = 0.1354$), which may be due to the fact that there were few patients who received combination treatment in this study.



Number at risk	0	20	40	60	80
+GCIRAE	44	24	14	11	2
–GCIRAE	48	16	13	11	5
–GCIRAE (+GC baseline)	6	2	2	1	1

Number at risk	0	20	40	60	80
+GCIRAE	44	33	25	21	4
–GCIRAE	48	24	17	16	8
–GCIRAE (+GC baseline)	6	2	2	1	1





Time (months) survivor (PFS) (95% CI)		
–GCIRAE		
	6	0.48 (0.33-0.61)
	12	0.40 (0.26-0.53)
	18	0.33 (0.20-0.46)
+GCIRAE		
	6	0.77 (0.62-0.87)
	12	0.64 (0.48-0.76)
	18	0.59 (0.43-0.72)

Time (months) survivor (OS) (95% CI)		
–GCIRAE		
	6	0.77 (0.62-0.87)
	12	0.62 (0.47-0.74)
	18	0.50 (0.35-0.63)
	24	0.44 (0.30-0.57)
+GCIRAE		
	6	0.95 (0.83-0.99)
	12	0.91 (0.78-0.96)
	18	0.82 (0.67-0.90)
	24	0.75 (0.59-0.85)

Figure 1. +GCIRAE was associated with better clinical outcome. (A) Kaplan–Meier curves demonstrating the PFS for the different subgroups. Median PFS for +GCIRAE, −GCIRAE, and −GCIRAE with baseline GC was 23.9, 5.2, and 0.9 months, respectively. (B) Kaplan–Meier curves demonstrating the OS for the three different subgroups. Median OS was 72.8, 17.7, and 1.4 months, respectively. (C and D) Percentage of patients who were progression-free (C) and alive (D) at 6, 12, 18, and 24 (only D) months in the two subgroups +GCIRAE and −GCIRAE, excluding −GCIRAE with baseline GC. Tables showing percentage of survivors with 95% confidence intervals. The tick marks indicate censored patients.
CI, confidence interval; GC, glucocorticoid; GCIRAE, glucocorticoid immune-related adverse event; OS, overall survival; PFS, progression-free survival.

+GCIRAE was correlated with better clinical outcome

When comparing Kaplan–Meier curves, we observed a longer median PFS for patients with +GCIRAE (23.9 months) compared with −GCIRAE (5.2 months) and −GCIRAE patients receiving systemic GCs within 30 days before ICIs start (0.9 months) (log-rank test $P = 0.0384$) (Figure 1A). A similar finding was observed in the Kaplan–Meier curves for OS, with a median OS of 72.8, 17.7, and 1.4 months for +GCIRAE, −GCIRAE, and for −GCIRAE patients receiving systemic GCs within 30 days before ICIs start, respectively (Figure 1B) (log-rank test $P = 0.00124$). In addition, we compared the proportion of progression-free and alive patients at 6, 12, 18, and 24 (only alive) months between +GCIRAE and −GCIRAE, excluding

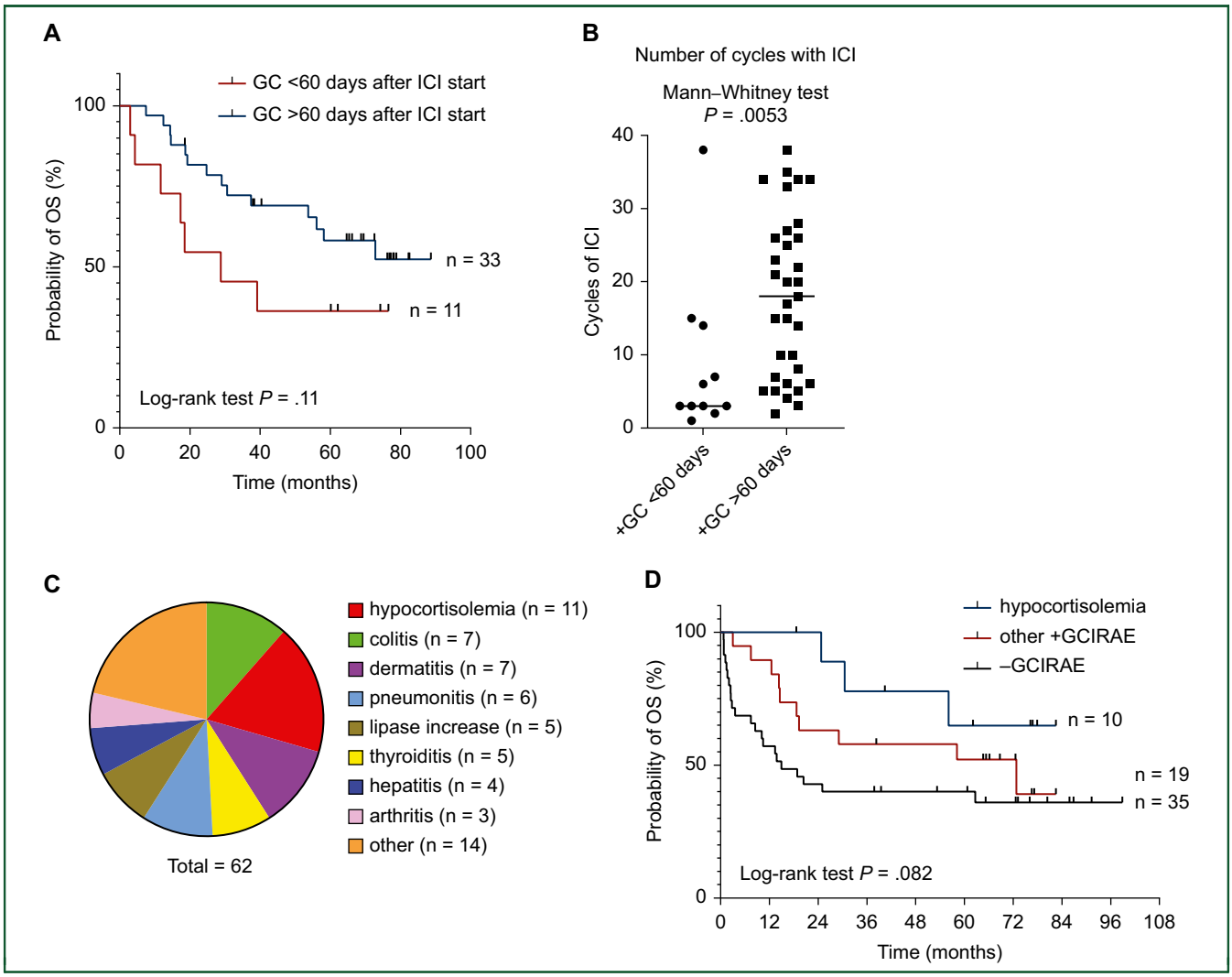


Figure 2. GC given within 60 days after ICI start may impact outcome negatively. (A) Kaplan—Meier curves demonstrating OS in +GCIRAE patients, subdivided into those who required GC for <60 days versus those who required GC for >60 days after ICI treatment start. (B) Cycles of ICI given in subgroups GC <60 days and GC >60 days after ICI treatment start. (C) Proportion of different types of GCIRAEs in the cohort. (D) Kaplan—Meier curves demonstrating OS for males with hypocortisolemia, other +GCIRAE and −GCIRAE including +GC baseline. The tick marks indicate censored patients.
GC, glucocorticoids; GCIRAE, glucocorticoid immune-related adverse event; ICI, immune checkpoint inhibitor; OS, overall survival; PFS, progression-free survival.

the third group who received GCs at baseline. The 95% CI for +GCIRAE and −GCIRAE do not overlap at 6 months regarding PFS and do not overlap at 12, 18, and 24 months regarding OS (Figure 1C and D).

Shorter ICIs duration correlated with worse clinical outcome in patients with +GCIRAE

The median time for initiation of IRAEs requiring systemic GCs treatment after ICIs therapy start was 146 days (range 17-661 days). Of the 44 patients developing +GCIRAE, 20 (45.5%) individuals ceased treatment because of toxicity, 11 (25%) patients due to either deep response or 2 years on therapy, 8 (18.2%) patients due to disease progression, and 1 (2.3%) patient lost at follow-up. In four (9%) patients, occurrence of toxicity in combination with a complete response contributed to the decision to stop therapy. A total of 11 of the 44 patients with +GCIRAE were given systemic GCs within 60 days after the start of ICI treatment and those subjects had an unfavorable OS compared with the 33 subjects starting systemic GCs later than 60 days after ICI therapy start (Figure 2A). The median OS was 28.8 months for those treated with GCs within 60 days whereas the median OS has not been reached for the other group. There was no difference between the two groups regarding prednisolone start dose, 40 mg (20-216 mg) and 40 mg (5-183 mg) ($P = 0.445$) and maximum dose prednisolone ($P = 0.363$) (Supplementary Table S1, available at https://doi.org/10.1016/j.iotech.2024.100713). The 11 patients with early +GCIRAE, however, received significantly fewer cycles of ICIs (Mann—Whitney test $P = 0.0053$; Figure 2B). A total of 5 of these 11 patients ended ICI treatment prematurely due to side-effects, versus only 1 patient due to progress.

The most common +GCIRAE was hypocortisolemia which correlated with improved clinical outcome

A total of 16 of 44 patients received GCs for more than one IRAE, generating in total 62 +GCIRAE (Supplementary Table S2, available at https://doi.org/10.1016/j.iotech.2

024.100713, Figure 2C). The most common +GCIRAE were hypocortisolemia ($n = 11$), dermatitis ($n = 7$), colitis ($n = 7$), and pneumonitis ($n = 6$) (Supplementary Table S2, available at https://doi.org/10.1016/j.iotech.2024.100713, Figure 2C). Hypocortisolemia occurred often later with a median time of 279 days after ICI start (43-562 days) and in 8/11 cases it was the first IRAE event. During the first years after introduction of ICI therapy in Sweden, patients developing hypocortisolemia were treated more aggressively regarding cortisone dose compared with current practice, which is shown in Supplementary Table S3, available at https://doi.org/10.1016/j.iotech.2024.100713. These patients received even higher doses of GCs than those with colitis and pneumonitis, and two of them interrupted ICI due to hypophysitis.

A total of 10 out of the 11 cases of ICI-related hypocortisolemia were evaluated by an endocrinologist. Detailed information about disease features and treatment details in 11 patients with hypocortisolemia are presented in Supplementary Table S4, available at https://doi.org/10.1016/j.iotech.2024.100713. The reason for hypocortisolemia was hypophysitis in seven cases, adrenal insufficiency in two cases and in two cases was undetermined. These diagnoses were based on hormonal and laboratory values, clinical symptoms, and imaging in some cases. Interestingly, all cases of hypocortisolemia, except for one, were in males. The median OS for male patients developing hypocortisolemia was still not reached at study cut-off, whereas for males with +GCIRAE other than hypocortisolemia and for those without any GCIRAE it was 72.8 and 15.0 months, respectively (P value = 0.082) (Figure 2D).

+GCIRAE Patients had longer survival even though this was not statistically significant after adjustment for immortal time bias

We first fit a crude model with the longitudinal GCIRAE as the only covariate which showed no significant effect for PFS or OS (Table 2). The following potential covariates were investigated in bivariate models together with GCIRAE (Supplementary Table S5, available at https://doi.org/10.1016/j.iotech.2024.100713), as motivated by clinical reasoning: age, sex, M1 stage, and LDH. As age, M1 stage, and LDH made at least a 5% impact on the effect of GCIRAE, it was decided to include them in a multivariate final Cox model (Supplementary Table S5, available at https://doi.org/10.1016/j.iotech.2024.100713, Table 2).

Table 2. Uni- and multivariate analysis using time dependent Cox model.

PFS			
Univariate	HR	95% CI	*P* value
GCIRAE (yes)	1.24	0.75-2.04	0.4
Multivariate	HR	95% CI	*P* value
Age	1.01	0.99-1.04	0.2
M1 stage (M1c+d)	1.92	1.20-3.05	0.005
Elevated LDH (yes)	2.05	1.27-3.31	0.003
GCIRAE (yes)	1.08	0.65-1.81	0.8
OS			
Univariate	HR	95% CI	*P* value
GCIRAE (yes)	0.89	0.49-1.60	0.7
Multivariate	HR	95% CI	*P* value
Age	1.03	1.00-1.06	0.024
M1 stage (M1c+d)	2.62	1.50-4.60	<0.001
Elevated LDH (yes)	1.93	1.08-3.43	0.026
GCIRAE	0.81	0.44-1.48	0.5

CI, confidence interval; GCIRAE, glucocorticoid immune-related adverse event; HR, hazard ratio; LDH, lactate dehydrogenase; OS, overall survival; PFS, progression-free survival.

The final multivariate Cox model shows that while the hazard rate for PFS increases significantly with increasing age, M1c+d stage or elevated LDH, GCIRAE status has no statistically significant effect on PFS (Table 2). For the OS, the final multivariate Cox model shows that while the hazard rate increases significantly with increasing age, M1c+d stage or elevated LDH, the decrease in OS seen in +GCIRAE patients is not statistically significant (Table 2). We note, however, that the estimated HR for GCIRAE is <1, which reflects a longer survival in +GCIRAE patients compared with −GCIRAE. The difference in OS seen between +GCIRAE and −GCIRAE patients in Figure 1 may hence be caused by immortal time bias.

A flexible parametric model was fit to PFS and OS to study the difference in survival over time which is not possible with the Cox model. The likelihood ratio test showed a tendency of significance for difference in effect of +GCIRAE at PFS over time ($P = 0.07$). Figure 3A shows the PFS from the flexible parametric model, as predicted for 70-year-old patients (which was the median age in our cohort) with LDH = 0 or 1 and/or M1 stage = 0 or 1. In this dataset, +GCIRAE patients have slightly longer PFS than −GCIRAE patients during the first year, even though this time trend was not statistically significant.

The likelihood ratio test gave no significant difference in effect of GCIRAE over time ($P = 0.31$) for OS. Figure 3B shows the survival from the flexible parametric model, as predicted for 70-year-old patients with LDH = 0 or 1 and/or M1 stage = 0 or 1. In summary, +GCIRAE patients survived slightly longer than −GCIRAE patients in our dataset, particularly, in an intermediate time range (6 months to 4 years), even though this time trend was not statistically significant. Similar figures for other values of age, LDH, and M1 stage conceptually give the same picture.

Baseline expression of Delta/Notch-like epidermal growth factor-related receptor, interleukin 8, transforming growth factor-α, and fibroblast growth factor 5 were associated with hypocortisolemia

Fifty-seven baseline plasma samples were used to measure the expression levels of 92 inflammatory proteins (Olink Inflammation panel). Of the 92 proteins, 12 were below the assay LOD in >50% of the samples and were therefore discarded, leaving 80 proteins for subsequent analyses. None of the proteins were associated with PFS but seven proteins [capsase-8 (CASP8), chemokine (C-C motif) ligand 3 (CCL3), Delta/Notch-like epidermal growth factor-related receptor (DNER), ENRAGE/S100A12, hepatocyte growth factor (HGF), interleukin 6 (IL-6), and IL-8] were associated with OS when comparing patients with >2 years OS versus patients with >6

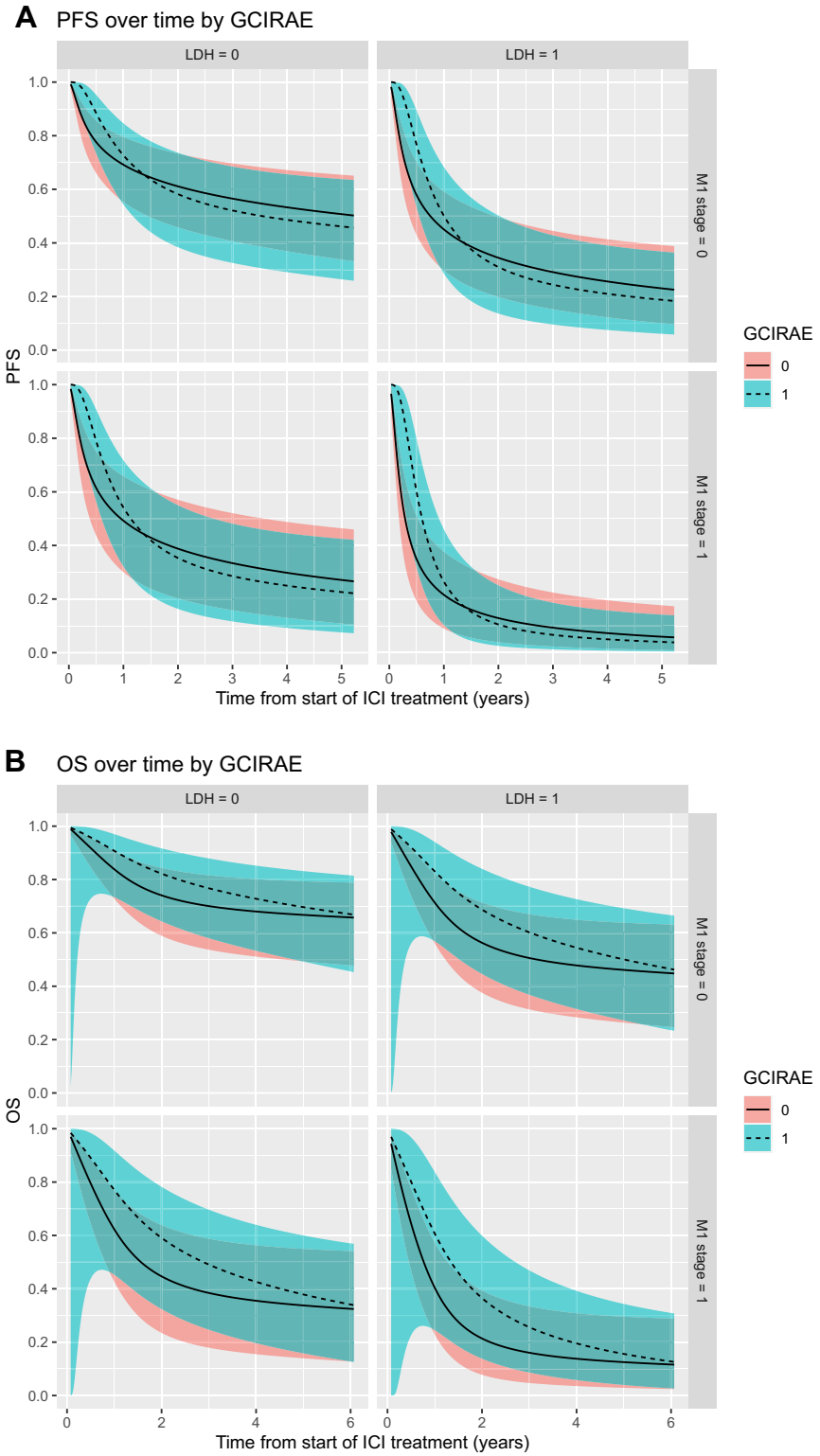
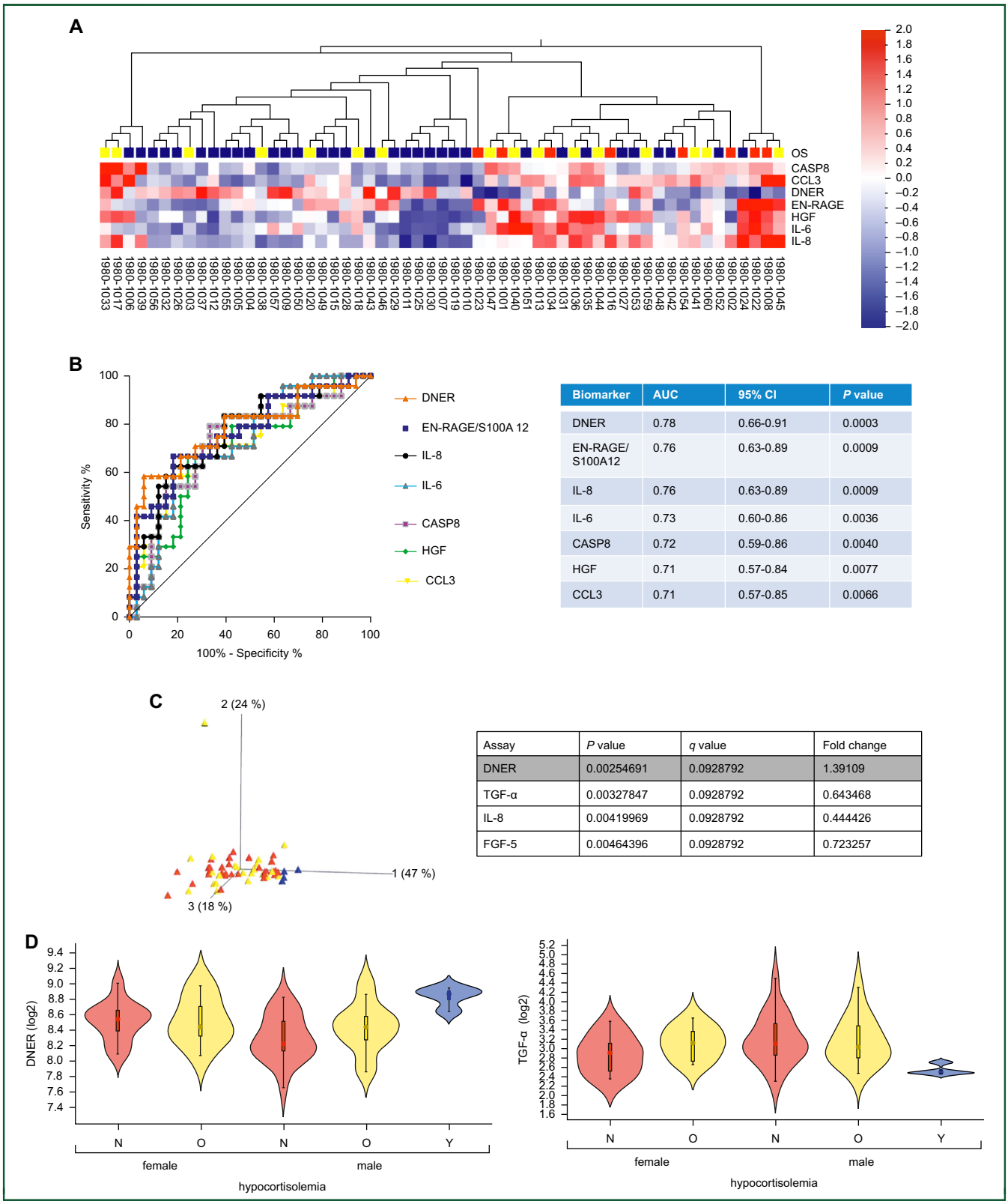


Figure 3. The figures show the progression free survival (PFS) (A) and overall survival (OS) (B) from the flexible parametric model, as predicted for 70-year-old patients with M1 stage 0 or 1 and/or LDH 0 or 1. M1 stage 0 = M1a+b; M1stage 1 = M1c+d; LDH 0 = normal; LDH 1 = elevated.
GCIRAE, glucocorticoid immune-related adverse event; ICI, immune checkpoint inhibitor; LDH, lactate dehydrogenase.

months <2 years and <6 months OS (adjusted *P* value; *q* value <0.1) (Figure 4A). We have compared the biomarkers with clinical factors. High IL-8 levels were associated with higher M1 stages and high S100A12 with higher levels of LDH (Supplementary Figure S1A and S1B, available at https://doi.org/10.1016/j.iotech.2024.100713). We used ROC curve estimates to evaluate the predictive capacity of these seven potential biomarkers to discriminate between patients dead



Biomarker	AUC	95% CI	*P* value
DNER	0.78	0.66-0.91	0.0003
EN-RAGE/ S100A12	0.76	0.63-0.89	0.0009
IL-8	0.76	0.63-0.89	0.0009
IL-6	0.73	0.60-0.86	0.0036
CASP8	0.72	0.59-0.86	0.0040
HGF	0.71	0.57-0.84	0.0077
CCL3	0.71	0.57-0.85	0.0066

Assay	*P* value	*q* value	Fold change
DNER	0.00254691	0.0928792	1.39109
TGF-α	0.00327847	0.0928792	0.643468
IL-8	0.00419969	0.0928792	0.444426
FGF-5	0.00464396	0.0928792	0.723257

Figure 4. Inflammation biomarkers associated with overall survival and hypocortisolemia. (A) Baseline levels of inflammation plasmatic proteins associated with OS when comparing patients with OS >2 years (blue) versus >6 months <2 years (yellow) and <6 months (red). (B) ROC curve and ROC-AUC with 95% CI displayed for DNER, ENRAGE/S100A12, IL-8, IL-6, CASP8, HGF, and CCL3. C) PCA plot showing clustering of samples based on baseline levels of four inflammation proteins (IL-8, TGF-α, FGF5, and DNER) associated with hypocortisolemia, restricted to male (*q* value <0.1). Hypocortisolemia (blue); other +GCIRAE (red); −GCIRAE (yellow). (D) Box plots showing DNER and TGF-α expression in females and males based on GCIRAE.

AUC, area under the curve; CASP8, capsase-8; CCL3, chemokine (C-C motif) ligand 3; CI, confidence interval; DNER, Delta/Notch-like epidermal growth factor-related receptor; GCIRAE, glucocorticoid immune-related adverse event; HGF, hepatocyte growth factor; IL-6, interleukin 6; IL-8, interleukin 8; OS, overall survival; PCA, principal component analysis; ROC, receiver operating characteristic; TGF-α, transforming growth factor-α.

or alive at 2 years after ICI therapy (Figure 4B). The predictive performances were as follows: DNER AUC 0.78 (95% CI 0.66-0.91), ENRAGE/S100A12 AUC 0.76 (95% CI 0.63-0.89), IL-8 AUC 0.76 (95% CI 0.63-0.89), IL-6 AUC 0.73 (95% CI 0.60-0.86), CASP8 AUC 0.72 (95% CI 0.59-0.86), HGF AUC 0.71 (0.57-0.84), CCL3 AUC 0.71 (95% CI 0.57-0.85). The results showed the highest predictive performance for DNER. To our knowledge this is the first time that DNER and S100 calcium binding protein A12 (ENRAGE/S100A12) are suggested as biomarkers for predicting OS after treatment with ICIs.

We could not identify any differentially expressed inflammation proteins when comparing +GCIRAE versus −GCIRAE in the 57 patients included in the biomarker analysis. Nevertheless, we found that male patients who developed hypocortisolemia had significantly lower expression of IL-8, transforming growth factor-α (TGF-α), and fibroblast growth factor 5 (FGF5) and higher expression of DNER compared with other +GCIRAE and −GCIRAE males (q value <0.1; Figure 4C and D), and both IL-8 and DNER were predictive for OS. Since no females with hypocortisolemia were included in the analysis we restricted this analysis to males.

DISCUSSION

This real-world population study was carried out in a cohort of 98 patients with metastatic CMM treated with ICIs to investigate the impact of +GCIRAE on treatment efficacy and whether +GCIRAE could be predicted by baseline plasmatic inflammation proteins.

In our cohort, patients developing +GCIRAE had a significantly longer OS compared with those who did not develop −GCIRAE. Nevertheless, when adjusting for immortal time bias, this survival advantage did not reach statistical significance even though a tendency for improved survival was maintained during the period between 6 months and 4 years after ICI start. The occurrence of +GCIRAE within 60 days after ICIs therapy start impacted prognosis negatively. In addition, a subgroup of subjects receiving GCs for cancer symptom control before ICIs had a dismal prognosis. The most common +GCIRAE was hypocortisolemia which mainly occurred among males and was strongly associated with favorable OS.

Some previous studies including patients with metastatic CMM have suggested that systemic GCs do not affect the efficacy of ICIs when they are used to treat IRAEs[18,19] whereas other studies suggested a potential negative effect of GCs on ICIs outcome.[17,23] A meta-analysis including 16 studies and 4045 patients observed that taking GCs during ICI therapy to treat IRAEs did not result in unfavorable OS.[24] Our findings suggest that the positive effect of immune activation may overcome the immunosuppressive effect of GCs. The OS advantage magnitude for +GCIRAE patients was attenuated after adjustment for immortal time bias suggesting that the survival advantage may at least partially be explained by longer exposure to ICIs.[25] Our dataset raises the concern that early prescription of GCs to treat IRAEs (within 60 days after ICIs start) may have a negative impact on the antitumoral immune response, but another possible explanation for this observation could be that ICI therapy was interrupted more prematurely in this patient subgroup (Figure 2B). In agreement with our findings, a retrospective multicenter study including individuals with advanced lung cancer treated with anti-PD-1 showed that ICIs interruption due to IRAEs significantly decreased OS.[26] Also, Maslov et al.[27] compared outcome of cancer patients treated with ICIs and using systemic GCs before and after 2 months after ICIs start. They found that early use of GCs either for IRAEs and non-IRAE indications had a deleterious impact on PFS and OS.[27] In another large multicenter retrospective study including 947 patients with advanced CMM treated with anti-PD-1 alone, the authors reported that early use of high dose of GCs was independently associated with unfavorable survival.[28] Taken together, these findings highlight the importance of carrying out prospective larger randomized studies comparing other types of more selective immunosuppressors as an alternative to GCs, such as IL-6 inhibitors, specially to treat IRAEs occurring early during ICIs.[29,30] High levels of IL-6 have been correlated with unfavorable outcomes in CMM patients treated with ICIs and IL-6 inhibition possibly increases the efficacy and tolerance of ICIs.[31]

We also observed that patients receiving GCs at baseline for palliative reasons had a very short OS. This small group of patients had a very advanced or resistant disease which probably explains the extremely poor outcome observed in this subgroup, rather than the tumoral immunosuppressive effects of GCs. Our data confirm the results of at least three other retrospective studies including lung cancer patients treated with ICIs where individuals receiving GCs during baseline for supportive reasons or to palliate cancer-related symptoms had worse outcome.[32-34] These data suggest that the reason for GC therapy is a relevant confounding factor. The aforementioned meta-analysis of Petrelli et al.[24] also showed that unfavorable OS was observed in subjects taking GCs for supportive reasons (HR = 2.5, 95% CI 1.41-4.43; P <0.01).

Hypocortisolemia occurred later compared with the overall +GCIRAE (279 days versus 146 days) and these patients received a median of 18 ICI cycles which together can be a part of the explanation for the good outcome. At the same time, the optimal number of cycles needed for each individual to achieve durable response is not known and some patients may be over-treated without any additional clinical effect. Clinical trials are needed to find the optimal duration of ICIs and avoid over-treatment. Development of late IRAEs and unnecessary costs may thereby be avoided. Ninety-one percent (10/11) of the patients developing hypocortisolemia were male. Our data are in accordance with the literature that reports a prevalence of ICI-related hypophysitis a little more than 10% and being 2-5 times more common in males.[35,36]

The occurrence of +GCIRAE could not be predicted by either the baseline clinical characteristics or by the proteomics analysis. This is in line with a recent publication by Nuñez et al. showing that no significant differences among

subgroups with and without IRAEs were observed when measuring the expression of 92 proteins (Olink inflammation panel) in 172 pretreatment serum samples (lung cancer and melanoma) while an increase of chemokine ligand 9 (CXCL9), CXCL10, CXCL11, and interferon-γ (IFN-γ) was observed early (within 2 weeks) on ICI treatment samples among subjects developing IRAEs.[37] These findings suggest that it may be more informative to compare pretreatment plasma/serum with samples taken early on treatment to find inflammatory biomarkers predicting risk for development of GCIRAE. We observed, however, a significantly lower baseline plasmatic expression of IL-8, TGF-α, FGF5 and higher baseline expression of DNER in male patients developing hypocortisolemia.

IL-8 is a well-known unfavorable prognostic biomarker in melanoma and TGF-α and FGF5 have been suggested to be poor prognostic molecular factors in other tumor types.[38-40] DNER is a less studied protein and has been shown in a mouse model to regulate IFN-γ secretion.[41] DNER, IL8, and S100A12 had the best performance in distinguishing patients dead or alive at 2 years after ICI therapy in the ROC analysis. The S100A12 protein is a mediator of inflammation secreted by neutrophils and has been associated with chronic inflammatory diseases. Pico de Coaña et al.[42] have shown that a higher neutrophil count before starting ICI was associated with a shorter PFS.

Our study's main limitation is that it is a retrospective, non-interventional, single center study, and the inherent bias related to this type of study must be considered. Data about start and maximal dose of GCs could be extracted from the medical records, but data on duration and cumulative dose of GCs was less reliable, as the date of discontinuation was not always stated. Nevertheless, our study is from an unselected real-world population that reflects real-life patients in clinical praxis. The ideal study design to address the best treatment to each specific IRAE are prospective randomized studies. They are, however, difficult to carry out and to interpret given the heterogeneity of IRAEs, of an individual's propensity for developing IRAEs, and of the specific mechanisms behind each IRAE including T-cell activation, and antibody-, cytokine- or complement-mediated IRAEs.[43]

The −GCIRAE was likely a more heterogeneous group, including individuals with no IRAEs, with low-grade IRAEs not demanding GCs and patients who could have used corticosteroids due to other reasons than IRAEs. Nevertheless, dividing the −GCIRAE group into further minor subgroups would not have added significant information to our analysis due to a lack of power. In addition, a retrospective grading of IRAE subjective reports by many different clinicians and defining whether diffuse symptoms were caused by ICIs or not, was not considered a reliable strategy. We have therefore chosen to rely on the prescription of GCs as an objective way to define a clinically significant IRAE. In the +GCIRAE group we observed that clinicians used different GC agents at different doses indicating that physicians' own experiences play an important role in the treatment decision, although guidelines are available. We do not, however, observe any differences in prednisolone dose equivalents start dose between patients who receive GCs earlier or later. Although we have observed interesting associations between outcome and +GCIRAE, we cannot conclude that there is a causal relationship between these events, since this is an observational study, and other factors may explain these findings.

By putting our data together with previous published studies, our findings contribute to increase awareness about the fact that GCs may be used to treat IRAEs when considered necessary, without significantly hampering the effect of ICIs. This may be especially important if GCs would increase the chances of keeping patients on ICI therapy. Systemic GCs should be very carefully used, however, with the lowest possible dose and the shortest possible duration, especially early during ICI therapy, since it may impact PFS and OS negatively. We can also conclude that other treatment strategies instead of ICIs, including best supportive care only, should be considered for patients with high tumor burden and impaired general health taking GCs for symptom control before ICI therapy start. Finally, the prognostic value of immune-related hypocortisolemia in individuals receiving ICIs and specific inflammation proteins as potential predictive biomarkers for IRAEs should be further investigated.

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DISCLOSURE

The authors have declared no conflicts of interest beyond those stated on the ICMJE conflict of interest forms.

REFERENCES

1. Robert C, Long GV, Brady B, et al. Nivolumab in previously untreated melanoma without BRAF mutation. *N Engl J Med.* 2015;372(4):320-330.
2. Robert C, Carlino MS, McNeil C, et al. Seven-year follow-up of the phase III KEYNOTE-006 study: pembrolizumab versus ipilimumab in advanced melanoma. *J Clin Oncol.* 2023;41(24):3998-4003.
3. Wolchok JD, Chiarion-Sileni V, Gonzalez R, et al. Long-term outcomes with nivolumab plus ipilimumab or nivolumab alone versus ipilimumab in patients with advanced melanoma. *J Clin Oncol*. 2022;40(2):127-137.
4. Davies H, Bignell GR, Cox C, et al. Mutations of the BRAF gene in human cancer. *Nature*. 2002;417(6892):949-954.

5. Larkin J, Ascierto PA, Dréno B, et al. Combined vemurafenib and cobimetinib in BRAF-mutated melanoma. *N Engl J Med.* 2014;371(20):1867-1876.
6. Long GV, Stroyakovskiy D, Gogas H, et al. Dabrafenib and trametinib versus dabrafenib and placebo for Val600 BRAF-mutant melanoma: a multicentre, double-blind, phase 3 randomised controlled trial. *Lancet*. 2015;386(9992):444-451.
7. Dummer R, Ascierto PA, Gogas HJ, et al. Encorafenib plus binimetinib versus vemurafenib or encorafenib in patients with BRAF-mutant melanoma (COLUMBUS): a multicentre, open-label, randomised phase 3 trial. *Lancet Oncol*. 2018;19(5):603-615.
8. Ascierto PA, Mandalà M, Ferrucci PF, et al. Sequencing of ipilimumab plus nivolumab and encorafenib plus binimetinib for untreated BRAF-mutated metastatic melanoma (SECOMBIT): a randomized, three-arm, open-label phase II trial. *J Clin Oncol*. 2023;41(2):212-221.
9. Weber J, Mandala M, Del Vecchio M, et al. Adjuvant nivolumab versus ipilimumab in resected stage III or IV melanoma. *N Engl J Med.* 2017;377(19):1824-1835.
10. Luke JJ, Rutkowski P, Queirolo P, et al. Pembrolizumab versus placebo as adjuvant therapy in completely resected stage IIB or IIC melanoma (KEYNOTE-716): a randomised, double-blind, phase 3 trial. *Lancet*. 2022;399(10336):1718-1729.
11. Patel SP, Othus M, Chen Y, et al. Neoadjuvant-adjuvant or adjuvant-only pembrolizumab in advanced melanoma. *N Engl J Med.* 2023;388(9):813-823.
12. Haanen J, Obeid M, Spain L, et al. Management of toxicities from immunotherapy: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2022;33(12):1217-1238.
13. Larkin J, Chiarion-Sileni V, Gonzalez R, et al. Combined nivolumab and ipilimumab or monotherapy in untreated melanoma. *N Engl J Med.* 2015;373(1):23-34.
14. Zhou X, Yao Z, Yang H, et al. Are immune-related adverse events associated with the efficacy of immune checkpoint inhibitors in patients with cancer? A systematic review and meta-analysis. *BMC Med*. 2020;18(1):87.
15. Amoroso V, Gallo F, Alberti A, et al. Immune-related adverse events as potential surrogates of immune checkpoint inhibitors' efficacy: a systematic review and meta-analysis of randomized studies. *ESMO Open*. 2023;8(2):100787.
16. Bjelaković G, Stojanović I, Stoimenov TJ, et al. Metabolic correlations of glucocorticoids and polyamines in inflammation and apoptosis. *Amino Acids*. 2010;39(1):29-43.
17. Eggermont AMM, Kicinski M, Blank CU, et al. Association between immune-related adverse events and recurrence-free survival among patients with stage III melanoma randomized to receive pembrolizumab or placebo: a secondary analysis of a randomized clinical trial. *JAMA Oncol*. 2020;6(4):519-527.
18. Attia P, Phan GQ, Maker AV, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. *J Clin Oncol*. 2005;23(25):6043-6053.
19. Indini A, Di Guardo L, Cimminiello C, et al. Immune-related adverse events correlate with improved survival in patients undergoing anti-PD1 immunotherapy for metastatic melanoma. *J Cancer Res Clin Oncol*. 2019;145(2):511-521.
20. Horvat TZ, Adel NG, Dang TO, et al. Immune-related adverse events, need for systemic immunosuppression, and effects on survival and time to treatment failure in patients with melanoma treated with ipilimumab at Memorial Sloan Kettering Cancer Center. *J Clin Oncol*. 2015;33(28):3193-3198.
21. Weber JS, Hodi SF, Wolchok JD, et al. Safety profile of nivolumab monotherapy: a pooled analysis of patients with advanced melanoma. *J Clin Oncol*. 2017;35(7):785-792.
22. Gershenwald JE, Scolyer RA. Melanoma staging: American Joint Committee on Cancer (AJCC) 8th edition and beyond. *Ann Surg Oncol*. 2018;25(8):2105-2110.
23. Romanski NA, Holmstroem RB, Ellebaek E, et al. Characterization of risk factors and efficacy of medical management of immune-related hepatotoxicity in real-world patients with metastatic melanoma treated with immune checkpoint inhibitors. *Eur J Cancer.* 2020;130:211-218.
24. Petrelli F, Signorelli D, Ghidini M, et al. Association of steroids use with survival in patients treated with immune checkpoint inhibitors: a systematic review and meta-analysis. *Cancers (Basel)*. 2020;12(3):546.
25. Kawai T, Taguchi S, Nakagawa T, et al. Impact of immune-related adverse events on the therapeutic efficacy of pembrolizumab in urothelial carcinoma: a multicenter retrospective study using time-dependent analysis. *J Immunother Cancer.* 2022;10(2):e003965.
26. Ksienski D, Wai ES, Croteau N, et al. Efficacy of nivolumab and pembrolizumab in patients with advanced non-small-cell lung cancer needing treatment interruption because of adverse events: a retrospective multicenter analysis. *Clin Lung Cancer.* 2019;20(1):e97-e106.
27. Maslov DV, Tawagi K, Kc M, et al. Timing of steroid initiation and response rates to immune checkpoint inhibitors in metastatic cancer. *J Immunother Cancer.* 2021;9(7):e002261.
28. Bai X, Hu J, Warner AB, et al. Early use of high-dose glucocorticoid for the management of irAE is associated with poorer survival in patients with advanced melanoma treated with anti-PD-1 monotherapy. *Clin Cancer Res*. 2021;27(21):5993-6000.
29. Dimitriou F, Hogan S, Menzies AM. Interleukin-6 blockade for prophylaxis and management of immune-related adverse events in cancer immunotherapy. *Eur J Cancer.* 2021;157:214-224.
30. Holmstroem RB, Nielsen OH, Jacobsen S, et al. COLAR: open-label clinical study of IL-6 blockade with tocilizumab for the treatment of immune checkpoint inhibitor-induced colitis and arthritis. *J Immunother Cancer.* 2022;10(9):e005111.
31. Hailemichael Y, Johnson DH, Abdel-Wahab N, et al. Interleukin-6 blockade abrogates immunotherapy toxicity and promotes tumor immunity. *Cancer Cell*. 2022;40(5):509-523.e6.
32. Skribek M, Rounis K, Afshar S, et al. Effect of corticosteroids on the outcome of patients with advanced non-small cell lung cancer treated with immune-checkpoint inhibitors. *Eur J Cancer.* 2021;145:245-254.
33. Fucà G, Galli G, Poggi M, et al. Modulation of peripheral blood immune cells by early use of steroids and its association with clinical outcomes in patients with metastatic non-small cell lung cancer treated with immune checkpoint inhibitors. *ESMO Open*. 2019;4(1):e000457.
34. Arbour KC, Mezquita L, Long N, et al. Impact of baseline steroids on efficacy of programmed cell death-1 and programmed death-ligand 1 blockade in patients with non-small-cell lung cancer. *J Clin Oncol*. 2018;36(28):2872-2878.
35. Mizukoshi T, Fukuoka H, Takahashi Y. Immune checkpoint inhibitor-related hypophysitis. *Best Pract Res Clin Endocrinol Metab*. 2022;36(3):101668.
36. Caturegli P, Di Dalmazi G, Lombardi M, et al. Hypophysitis secondary to cytotoxic T-lymphocyte-associated protein 4 blockade: insights into pathogenesis from an autopsy series. *Am J Pathol*. 2016;186(12):3225-3235.
37. Nuñez NG, Berner F, Friebel E, et al. Immune signatures predict development of autoimmune toxicity in patients with cancer treated with immune checkpoint inhibitors. *Med.* 2023;4(2):113-129.e7.
38. Sanmamed MF, Carranza-Rua O, Alfaro C, et al. Serum interleukin-8 reflects tumor burden and treatment response across malignancies of multiple tissue origins. *Clin Cancer Res*. 2014;20(22):5697-5707.
39. Kleeff J, Friess H, Berberat PO, et al. Pancreatic cancer-new aspects of molecular biology research. *Swiss Surg*. 2000;6(5):231-234.
40. Zhao T, Qian K, Zhang Y, et al. High expression of FGF5 is an independent prognostic factor for poor overall survival and relapse-free survival in lung adenocarcinoma. *J Comput Biol*. 2020;27(6):948-957.
41. Ballester-López C, Conlon TM, Ertüz Z, et al. The Notch ligand DNER regulates macrophage IFNγ release in chronic obstructive pulmonary disease. *EBioMedicine*. 2019;43:562-575.
42. Pico de Coaña Y, Wolodarski M, van der Haar Àvila I, et al. PD-1 checkpoint blockade in advanced melanoma patients: NK cells, monocytic subsets and host PD-L1 expression as predictive biomarker candidates. *Oncoimmunology.* 2020;9(1):1786888.
43. Postow MA, Sidlow R, Hellmann MD. Immune-related adverse events associated with immune checkpoint blockade. *N Engl J Med*. 2018;378(2):158-168.