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The baseline circulating immunophenotype characteristics associate with PD(L)-1 targeted treatment response, irae onset, and prognosis

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More promising, effective, and less-invasive biomarkers for PD(L)-1 targeted responses, immune-related adverse events (irAEs), and prognosis are being explored. We conducted a single-center retrospective study in pan-cancer patients with anti-PD(L)-1 monotherapy. Observational endpoints included treatment response, prognosis, and irAEs. Peripheral blood immunophenotypes were analyzed by Flow Cytometry. 104 patients were enrolled. Higher pretreatment percentages of $CD3^+CD4^+$ Th cells were associated with both responses (HR: 6.170, $P = 0.034$) and prognosis (HR: 1.930, $P = 0.022$). The higher baseline percentage of $CD16^+CD56^+$ NK cells was positively correlated with response (HR: 3.730, $P = 0.050$) and negatively related to irAEs (HR: 0.460, $P = 0.012$). Decreased pretreatment $CD3^+$ T cell counts were related to more irAEs (HR: 0.970, $P = 0.026$), while the percentage of $CD3^+$ T cells was negatively associated with prognosis (HR: 1.930, $P = 0.022$). The higher baseline cell counts of $CD3^+CD8^+$ CTL, $CD19^+$ B, and the percentage of $CD19^+$ B cells might be related to more irAEs ($P < 0.05$). Significant correlation between duration of treatment (DOT) and prognosis, irAE and outcome was also confirmed ($P < 0.0001$). Our findings confirmed multiple baseline circulating immunophenotype characteristics were related to PD(L)-1 targeted response, irAE onset, and prognosis.

Keywords PD(L)-1 inhibitor, Monotherapy, Response, IrAE, Prognosis, Biomarker

Abbreviations

PD-1	Programmed cell death protein-1
PD-L-1	Programmed death ligand-1
irAEs	Immune related adverse events
TTP	Time to progression
BMI	Body mass index
PLR	Platelet-to-lymphocyte ratio
DOT	Duration of therapy
SITC	Society for immunotherapy of cancer
NCCN	National comprehensive cancer network
ASCO	American society of clinical oncology
ESMO	European society for medical oncology

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uc-irAEs	unclassified irAEs
TMB	Tumor mutational burden
dMMR	Deficient mismatch repair
MSI-H	Microsatellite instability-high
ICI	Immune checkpoint inhibitor
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
TBIL	Total bilirubin
DBIL	Direct bilirubin
TBA	Total bile acids
ALP	Alkaline phosphatase
GGT	Gamma-glutamyl transferase
LDH	Lactate dehydrogenase
ALB	Albumin
GLB	Globulin
Cr	Creatinine
BUN	Blood urea nitrogen
UA	Uric acid
NLR	Neutrophil-to-lymphocyte ratio
LMR	Lymphocyte-to-monocyte ratio
SII	Systemic immune-inflammation index
CR	Complete response
PR	Partial response
SD	Stable disease
PD	Progressive disease

PD(L)-1 inhibitors have been extensively used and have revolutionized the treatment of various cancer types[1]. However, only a fraction of patients may benefit from anti-PD(L)-1-based therapies and achieve long-lasting survival[2]. Considering the selective efficacy and frequent but sometimes life-threatening toxicities of anti-PD(L)-1 antibodies, the determination of accurate biomarkers has significant value for maximizing therapeutic responses and minimizing irAEs[3,4]. Robust biomarkers with clinical feasibility for individual populations remain sparse[5].

To date, the US Food and Drug Administration has approved four biomarkers as companion or supplementary diagnostics, including programmed death ligand 1 (PD-L1) expression, tumor mutational burden (TMB), deficient mismatch repair (dMMR) and microsatellite instability-high (MSI-H)[6,7]. In addition, numerous studies have supported a strong relationship between immune checkpoint inhibitor (ICI) treatment response and irAE onset across a variety of solid tumors, indicating that biomarkers for response are also valuable for irAE prediction[8-10].

Previous studies have shown that demographic factors might be linked to ICI response and toxicities. More responders to ICI treatment were observed in male patients[11]. In contrast to efficacy, any grade or higher grade of irAEs were more likely to develop in females[12]. Prior evidence supports that obesity (BMI ≥ 25 kg/m^2) could be a predictive biomarker for a favorable response, prognosis, and more irAEs during ICI treatment in stage IV cancer patients with melanoma, non-small cell lung cancer, and kidney cancer, especially for male patients with BMI ≥ 30 kg/m^2 [13,14]. While this association was inconsistent in other observations, several studies showed that no relationship existed between BMI and response and prognosis[15]. Eastern Cooperative Oncology Group (ECOG) performance status is another critical factor that affects host immunity. It is usually difficult for patients with an ECOG score of 2 or higher to benefit from ICI therapy and achieve prolonged survival[16,17]. Nonetheless, the systemic analysis of the interplays among host demographics, anti-PD(L)-1 response, irAE, and prognosis remains lacking.

In recent years, several less-invasive blood-based indicators for response and irAEs have been investigated in various cancer types with PD(L)-1 targeted immunotherapy[7]. These biomarkers included circulating tumor DNA, organ-nonspecific autoantibodies, T cell subpopulation count, cell type ratios (NLR, PLR, LMR), PD-1 on peripheral blood CD4$^+$ T cells, cytokines/chemokines (IL-6, IL-8, IL-10), and so on[18-20]. Although easier to obtain, these specific blood biomarkers demonstrate limited efficacy and controversial potency, which need rigorous validation[5,18,20]. Moreover, hematological immunophenotypic biomarkers with completely or partially predictive effects for anti-PD(L)-1 responses, irAEs, and prognosis are being pursued.

This study aimed to systematically assess the role of baseline demographic features and multiple peripheral blood characteristics in anti-PD(L)-1 monotherapy treatment responses, irAEs, and prognosis. Leveraging these findings, we hope to provide more information for immunophenotypic biomarker exploration and pave the way for future comprehensive integration predictive models.

Materials and methods

Patient enrollment and ethical considerations

This retrospective study enrolled adult patients treated with anti-PD(L)-1 monotherapy. It has been approved by the Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University & Henan Cancer Hospital (grant number 2021-233-001) and registered with ClinicalTrials. gov, NCT06693440. The requirement for written informed consent was waived. All the eligible patients received at least one dose of anti-PD antibody therapy. We excluded patients with anti-PD(L)-1-based combination therapy, rapid tumor progression, active

infection, or a history of systemic glucocorticoid or immunosuppressive treatment at the time of data collection, which might affect the laboratory results.

Data collection

All patients' baseline demographics (including age, sex, ECOG score, BMI, smoke, alcohol, stage, metastatic sites, line of therapy, treatment cycle, type of anti-PD(L)-1 antibody, and tumor origin), clinical characteristics (including tests of peripheral hematological immunophenotype, blood routine, and biochemistry profile), treatment processes, irAEs, and survival time were collected and recorded for analysis. Biochemistry profile encompassed alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), direct bilirubin (DBIL), total bile acids (TBA), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), albumin (ALB), globulin (GLB), creatinine (Cr), blood urea nitrogen (BUN), uric acid (UA) and C-reactive protein (CRP). Cell type ratios consisted of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), lymphocyte-to-monocyte ratio (LMR), and systemic inflammation index (SII) were calculated. The percentage and cell count of the various hematological lymphocyte subpopulations were quantified using flow cytometry and analyzed as the immunophenotypes.

Flow cytometry analysis of blood immunophenotypes

Peripheral whole blood samples were collected in EDTA tubes at baseline, used directly for flow cytometry staining, and analyzed in the GMP laboratory of the Immunotherapy Department. For each tube, appropriate amounts of fluorochrome-conjugated mono-clonal antibody reagents were added to 100μL blood sample in sequence and then vortexed gently. Cells were stained with the following surface antibodies to determine the cell lineage (T-B-NK tube): anti-CD45 PerCP-Cy™5.5 (BD bioscience, Cat.No.652803), anti-CD3 PE/Cyanine7 (Biolegend, Cat.No.344816), anti-CD4 PE (Biolegend, Cat.No.317410), anti-CD8a FITC (Biolegend, Cat. No.301050), anti-CD19 APC (Biolegend, Cat. No.302212), anti-CD16 PE (Biolegend, Cat.No.360704), anti-CD56 PE (Biolegend, Cat.No.362508), anti-CD4 FITC (Biolegend, Cat.No.300538), anti-CD25 PE (Biolegend, Cat.No.302606), anti-CD127 APC (Biolegend, Cat.No.351316). Samples were acquired by FACS CantoII (BD Biosciences) within 2 h after being stained. The data was analyzed with FlowJo software (Tree Star, Inc.).

Endpoints and assessments

Treatment response was categorized as complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD). Patients with the disease control (CR + PR + SD) were classified as responders. In parallel, patients with PD were classified as non-responders. Time to progression (TTP) was defined as the time from the first anti-PD antibody injection to disease progression, consisting of either radiologic progression or subsequent anticancer therapy (including systemic therapy, radiotherapy, or surgery) or death. Duration of therapy (DOT) was defined as the time from the first anti-PD(L)-1 antibody infusion to drug withdrawal, drug discontinuation, first disease progression, or death. The exploration of prognostic factors was carried out between the progression and non-progression groups according to the cut-off follow-up date.

The primary endpoints were treatment response and TTP, which investigators assessed according to the Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 and Immunotherapy Response Evaluation Criteria in Solid Tumors (iRECIST) criteria. IrAEs were set as secondary endpoints, defined and graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0.

Statistical analysis

Continuous variables were assessed using independent t-tests, while categorical variables were evaluated using Pearson's chi-square analysis. Data were presented as mean ± SEM, where appropriate. Survival estimates were derived using the Kaplan-Meier method, and survival curves were compared across patient groups via the log-rank test. The Cox and Logistic regression analyses were used to ascertain predictive indicators for response and irAE/prognosis, respectively. Variables that demonstrated statistical significance ($P < 0.05$) in univariate analysis or those approaching significance ($P < 0.01$) were selected for inclusion in multivariate regression analysis. Statistical significance was defined as a p-value of less than 0.05 from two-tailed tests. All analyses were conducted utilizing SPSS Statistics 22.0 (IBM, Armonk, NY, USA), and figures were generated by Prism 9.0 (GraphPad Software, San Diego, California, USA). The overlapping patterns of features in the uc-irAE distribution parts were determined using UpSet plots generated with the UpSetR package 1.4.0.

Results

Patients' characteristics, treatment responses, and overall incidence of IrAEs

From December 2017 to May 2023, 104 patients were enrolled and analyzed, including 48 females and 56 males. The median age of the patients was 58 (range 20–88) years. 41 patients had an ECOG score of 0, 57 had a score of 1, and the remaining 6 had a score of 2. The cancer types included 44 melanomas, 17 lung cancers, 19 digestive system malignancies, 8 gynecological cancers, and 16 other tumors. Disease stages were categorized as early, middle, and advanced, accounting for 3, 37, and 64 patients, respectively. Among them, 26 had no metastatic lesions, 38 had a single metastatic lesion, and 40 had two or more metastatic lesions. 32 patients received PD(L)-1 inhibitor as adjuvant therapy, 30 as first-line therapy, 16 as maintenance therapy, and 26 as second or later-line therapy. Anti-PD antibodies included Nivolumab, Pembrolizumab, Durvalumab, Atezolizumab, Tislelizumab, Sintilimab, Toripalimab, and Camrelizumab.

The most commonly used anti-PD(L)-1 antibodies were Toripalimab, Sintilimab, and Pembrolizumab, accounting for 36%, 20%, and 16% of patients, respectively. The median treatment cycle was 8 (range 2–67), and 52% of the patients received more than ten cycles of anti-PD(L)-1 antibody infusion. Among these patients, 46 patients responded to treatment, while 36 patients did not exhibit a response. Meanwhile, irAEs were reported

in 78 patients, compared to 26 patients who did not experience any grade of irAEs. The details of patient demographics, PD(L)-1 inhibitor exposure, treatment responses, and irAEs are summarized and delineated in Table 1.

Univariate analysis of baseline demographics and hematological characteristics correlated with treatment responses, IrAEs, or prognosis

Anti-PD(L)-1 treatment cycles showed a significant correlation with responses, irAEs, and survival outcomes (Fig. 1A, $P<0.001$, Fig. 1B and C, $P<0.05$). The line of therapy was relevant to responses and prognosis (Fig. 1D, $P<0.01$, Fig. 1F, $P<0.001$) but not irAEs (Fig. 1E, $P>0.05$). Alcohol intake might affect both treatment responses and irAE occurrence (Fig. 1G and H, $P<0.05$) but not affect the outcomes (Fig. 1I, $P>0.05$). Sex was associated with irAE occurrence (Fig. 1K, $P<0.01$) but not related to response and prognosis (Fig. 1J and L, $P>0.05$). Patients with different ECOG scores showed diverse treatment responses (Fig. 1M, $P<0.05$) but similar irAE occurrence and survival outcomes (Fig. 1N and O, $P>0.05$). The agents of anti-PD antibody and cancer type revealed no statistical disparities in response, irAE, and prognosis (Fig. 1P and U, $P>0.05$).

More irAEs were reported in patients with higher baseline levels of circulating $CD45^+$ cell counts, $CD3^+$ T cell counts, $CD3^+CD4^+$ Th cell counts, $CD19^+$ B cell counts, and $CD4^+/CD8^+$ T cell ratio (Fig. 2A and E, $P<0.05$). However, the reverse tendency was observed between a higher percentage of $CD3^+CD8^+$ CTL and irAE onset (Fig. 2F, $P<0.05$). As for blood routine tests, the higher baseline lymphocytes, platelets, and LMR also showed a relationship with more irAEs (Fig. 2G and I, $P<0.05$). Additionally, no significant differences between responders and non-responders were identified in the rest of the 13 parameters of immunophenotype and blood test (Fig. 2J and V, $P>0.05$). No correlations were found between the biochemical profiles and responses (Supplementary Fig. 1, $P>0.05$).

In addition, we scrutinized the baseline characteristics of peripheral blood immunophenotypes, blood routine tests, and biochemistry profiles between the response and non-response, the progression and non-progression groups. No discernible alterations were observed among these parameters across different groups (Supplementary Fig. 2-Supplementary Fig. 4, $P>0.05$).

Multivariate analysis of baseline features affecting response, IrAE, and prognosis

Further, we conducted multivariate regression analyses. Smoking demonstrated an association with the response in both univariate and multivariate analysis (HR: 83091.640, 95% CI 26.710-258477780, $P=0.006$). Besides, sex preference was found in irAE occurrence, and female patients were more likely to experience irAEs (HR: 31.510, 95% CI 2.120-467.520, $P=0.012$). ECOG might be associated with response in univariate analysis and associated with irAE in multivariate analysis (HR: 18.580, 95% CI 2.440-141.180, $P=0.005$). We adjusted the results of the above-mentioned three demographic parameters in the multivariate analysis due to high deviation.

Cox analysis indicated that BMI, PLR, and the percentage of $CD3^+CD4^+$ Th cells were independent factors associated with treatment response (Fig. 3A, $P<0.05$). Higher baseline BMI was related to poorer response (HR: 0.070, 95% CI 0.000-0.910, $P=0.042$). High levels of pretreatment PLR (HR: 1.060, 95% CI 1.000-1.120, $P=0.041$), the percentage of $CD3^+CD4^+$ Th cell (HR: 6.170, 95% CI 1.150–33.730, $P=0.034$) and $CD16^+CD56^+$ NK cells (HR: 3.730, 95% CI 1.000-13.980, $P=0.050$) were associated with positive responses.

Moreover, Logistic regression analysis certified that $CD3^+$ T cell counts, $CD3^+CD8^+$ CTL cell counts, $CD19^+$ B cell counts, and the percentages of $CD19^+$ B and $CD16^+CD56^+$ NK cells were independent factors associated with irAE onset (Fig. 3B, $P<0.05$). Patients with higher baseline cell counts of $CD3^+CD8^+$ CTL (HR: 1.030, 95% CI 1.000-1.050, $P=0.048$) and $CD19^+$ B (HR: 1.050, 95% CI 1.010–1.080, $P=0.004$) were more likely to experience irAEs. In addition, lower baseline levels of $CD3^+$ T cell counts (HR: 0.970, 95% CI 0.940-1.000, $P=0.026$), the percentage of $CD19^+$ B (HR: 0.279, 95% CI 0.120–0.650, $P=0.003$) and $CD16^+CD56^+$ NK cells (HR: 0.460, 95% CI 0.250–0.840, $P=0.012$) were also associated with more irAEs. Of note, the percentage of $CD16^+CD56^+$ NK cells positively related to the response but negatively associated with irAEs.

In terms of prognosis, Logistic regression analysis demonstrated that the percentage of $CD3^+$ T and $CD3^+CD4^+$ Th cells were independent factors associated with outcomes (Fig. 3C, $P<0.05$). The percentage of $CD3^+CD4^+$ Th cells was positively correlated with prognosis (HR: 1.930, 95% CI 1.100–3.400, $P=0.022$). On the contrary, the percentage of $CD3^+$ T cells was a negative correlation factor for prognosis (HR: 0.350, 95% CI 0.150–0.810, $P=0.015$). Notably, a higher percentage of pretreatment $CD3^+CD4^+$ Th cells was associated with both positive responses and outcomes.

Adverse events distributions and the correlation with treatment responses

Among the 104 cases, 146 irAEs were reported in 78 (75%) patients. The irAEs included 51 (35%) endocrine, 33 (23%) hepatic, 26 (18%) pancreatic, 15 (10%) dermatologic, 11 (7%) renal, 6 (4%) cardiovascular, and 4 (3%) pulmonary toxicities. Of the 78 patients, 57 (73%) had grade 1 irAEs, 17 (22%) had grade 2 irAEs, and the remaining 4 (5%) had grade 3 or higher irAEs. No grade 5 irAEs were observed. Twelve of 78 cases with irAEs received glucocorticoid intervention, and the prevalence of patients requiring steroid administration was 15%. Single-system irAEs were presented in 29 (37%) patients, and multi-system irAEs were observed in 49 (63%) patients. The details of the distribution of irAEs are shown in Fig. 4A and B.

Apart from the classified irAEs, we observed a series of treatment-emerging adverse events that could not be diagnosed or classified by the current irAE terms according to the SITC/ASCO/ESMO/NCCN standards or criteria versions before 2024 [21–24]. We defined these toxicities as unclassified irAE (uc-irAE), which included but not limited to ALP, GGT, TBA, LDH, Creatine Kinase-MB, α-Hydroxybutyrate dehydrogenase, cholesterol, triglyceride, low-density lipoprotein, Apolipoprotein A (Apo-A), Apo-B and UA. These uc-irAEs occurred in 96 (92%) of 104 patients, and 8 patients did not experience any uc-irAE. The overlapping details of uc-irAEs and

	Baseline N (%)	Response N (%)	Non-response N (%)	Not-evaluated N (%)	P value	irAE N (%)	Non-irAE N (%)	P value
Age, years								
< 60	53 (51)	19 (41)	24 (67)	10 (45)	0.0765	39 (50)	14 (54)	0.8223
≥ 60	51 (49)	27 (59)	12 (33)	12 (55)		39 (50)	12 (46)	
Sex								
Male	56 (54)	24 (52)	22 (61)	10 (45)	0.3828	36 (46)	20 (77)	0.0070
Female	48 (46)	22 (48)	14 (39)	12 (55)		42 (54)	6 (23)	
ECOG score								
0	41 (39)	10 (22)	18 (50)	13 (59)	0.0444	27 (35)	14 (54)	0.1310
1	57 (55)	31 (67)	18 (50)	8 (36)		45 (57)	12 (46)	
2	6 (6)	5 (11)	0 (0)	1 (5)		6 (8)	0 (0)	
BMI								
Low	6 (6)	2 (4)	2 (6)	2 (9)	> 0.9999	5 (6)	1 (4)	0.4102
Normal	51 (49)	22 (48)	17 (47)	12 (55)		35 (45)	16 (61)	
High	47 (45)	22 (48)	17 (47)	8 (36)		38 (49)	9 (35)	
Smoke								
Yes	35 (34)	15 (33)	14 (39)	6 (27)	0.4968	23 (29)	12 (46)	0.1516
No	69 (66)	31 (67)	22 (61)	16 (73)		55 (71)	14 (54)	
Alcohol								
Yes	25 (24)	8 (17)	13 (36)	4 (18)	0.0466	15 (19)	10 (38)	0.0636
No	79 (76)	38 (83)	23 (64)	18 (82)		63 (81)	16 (62)	
Stage								
Early	3 (3)	0 (0)	1 (3)	2 (9)	0.1489	2 (3)	1 (4)	0.6937
Middle	37 (36)	7 (15)	11 (31)	19 (86)		29 (37)	8 (31)	
Advanced	64 (61)	39 (85)	24 (66)	1 (5)		47 (60)	17 (65)	
Metastatic sites								
0	26 (25)	2 (4)	6 (16)	18 (81)	0.2299	19 (24)	7 (27)	0.9591
1	38 (37)	20 (43)	15 (42)	3 (14)		29 (37)	9 (35)	
≥ 2	40 (38)	24 (52)	15 (42)	1 (5)		30 (39)	10 (38)	
Line of therapy								
Adjuvant	32 (31)	1 (2)	9 (25)	22 (100)	0.0033	24 (31)	8 (32)	0.8802
First line	30 (29)	15 (33)	15 (42)	0 (0)		21 (27)	9 (33)	
Maintenance	16 (15)	11 (24)	5 (14)	0 (0)		13 (17)	3 (12)	
Second or later line	26 (25)	19 (41)	7 (19)	0 (0)		20 (25)	6 (23)	
Treatment exposure								
≤ 5	30 (29)	8 (17)	19 (53)	3 (14)	0.0004	19 (24)	11 (42)	0.0240
6–10 11–19	20 (19) 36 (35)	8 (17) 16 (36)	9 (25) 6 (16)	7 (32) 10 (45)		15 (19)	9 (35) 5 (19)	
≥ 20	18 (17)	14 (30)	2 (6)	2 (9)		27 (35) 17 (22)	1 (4)	
Anti-PD(L)1 antibody								
Nivolumab	13 (12)	7 (15)	5 (14)	1 (5)	0.7869	8 (10)	5 (19)	0.7070
Pembrolizumab	17 (16)	9 (20)	6 (17)	2 (8)		13 (17)	4 (15)	
Durvalumab	2 (2)	1 (2)	0 (0)	1 (5)		2 (3)	0 (0)	
Atezolizumab	1 (1)	1 (2)	0 (0)	0 (0)		1 (1)	0 (0)	
Tislelizumab	12 (12)	7 (15)	4 (11)	1 (5)		7 (9)	5 (19)	
Sintilimab	21 (20)	12 (26)	8 (22)	1 (5)		17 (22)	4 (15)	
Toripalimab	37 (36)	9 (20)	12 (33)	16 (72)		29 (37)	8 (32)	
Camrelizumab	1 (1)	0 (0)	1 (3)	0 (0)		1 (1)	0 (0)	
Cancer origination								
Melanoma	44 (43)	12 (26)	18 (50)	14 (64)	0.1525	33 (42)	11 (42)	0.9786
Lung	17 (16)	8 (17)	7 (19)	2 (9)		12 (15)	5 (19)	
Digestive	19 (18)	8 (17)	5 (14)	6 (27)		14 (18)	5 (19)	
Gynecologic	8 (8)	6 (14)	2 (6)	0 (0)		6 (8)	2 (8)	
Others	16 (15)	12 (26)	4 (11)	0 (0)		13 (17)	3 (12)	

Table 1. Baseline demographics, treatment responses and IrAEs ($n = 104$).

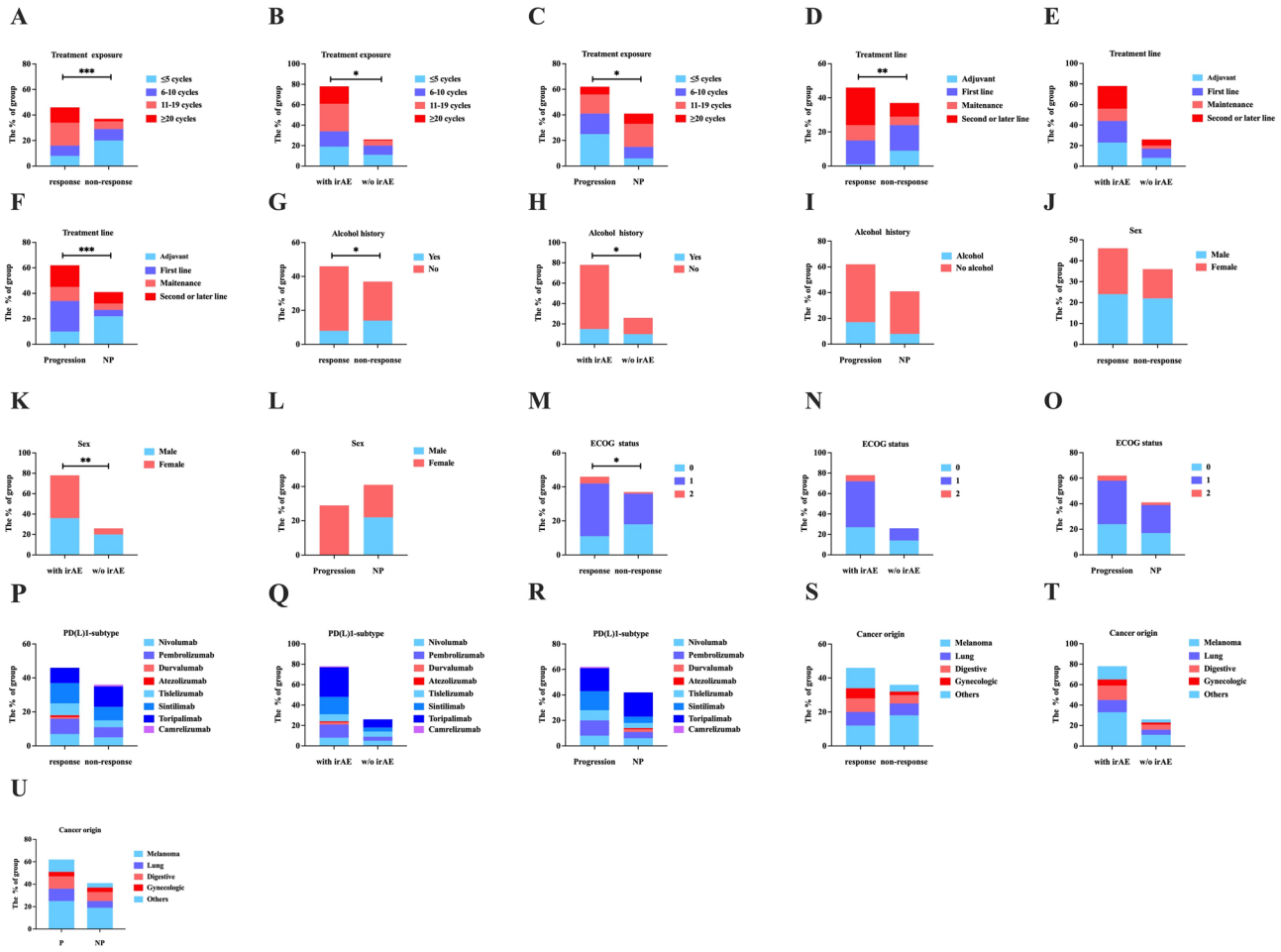


Fig. 1. Baseline demographics correlated with treatment response, irAEs, or disease progression. (**A**-**C**) Anti-PD(L)-1 treatment cycles were divided into groups of ≤ 5 cycles, 5–10 cycles, 11–19 cycles, and ≥ 20 cycles infusions. (**D**-**F**) Lines of therapy were compared among the adjuvant, first-line, maintenance, and second or later therapy groups. (**G**-**I**) Comparison between patients with and without alcohol history. (**J**-**L**) Sex differences were analyzed among different groups. (**M**-**O**) Comparison of different ECOG performance statuses. (**P**-**R**) Different anti-PD(L)-1 inhibitors were compared. (**S**-**U**) The distribution of cancer origins was assessed. Categorical variables were compared using the Chi-square test. Statistical differences were indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Abbreviations: w/o irAE, without irAE; P, progression; NP, non-progression.

irAEs are demonstrated in Fig. 4C. Among the 96 patients with uc-irAEs, 66 achieved a treatment response, and 74 experienced single or multiple definite irAEs.

Patients with and without irAEs demonstrated significant differences between response and non-response groups (Fig. 4D, $P < 0.001$). Furthermore, we analyzed the relationship between patient characteristics and uc-irAE occurrence. These characteristics included treatment response, best of response (BOR), presence or absence of irAEs, irAE systems, irAE grades, glucocorticoid administration, sex, age, stage, line of therapy, treatment exposure, and cancer types. The detailed analysis is presented in Supplementary Table 1. By comparison, we found that uc-irAEs were only associated with treatment response (Fig. 4E, $P < 0.05$).

The interactions among DOT, response, IrAE, and TTP

At the data cut-off date on July 31, 2024, the median follow-up was 56.9 months (95%CI, 41.3 to 72.6). A statistical correlation was observed between DOT and TTP (Fig. 5A, $R^2 = 0.2916$, $P < 0.0001$). The median TTP was 24.8 months (95%CI, 12.7 to 49.7) and 4.9 months (95%CI, 4.0 to 5.8) for responders and non-responders, respectively (Fig. 5B, hazard ratio: 0.1741, $P < 0.0001$). Subgroup analysis indicated that the median TTP in CR/PR or SD patients was significantly extended compared to those patients with PD (Figs. 5C and 18.2 months vs. 23.7 months vs. 4.8 months, $P < 0.0001$). However, CR/PR patients did not exhibit improved TTP compared to SD patients (Figs. 5C and 18.2 months vs. 23.7 months, $P > 0.05$).

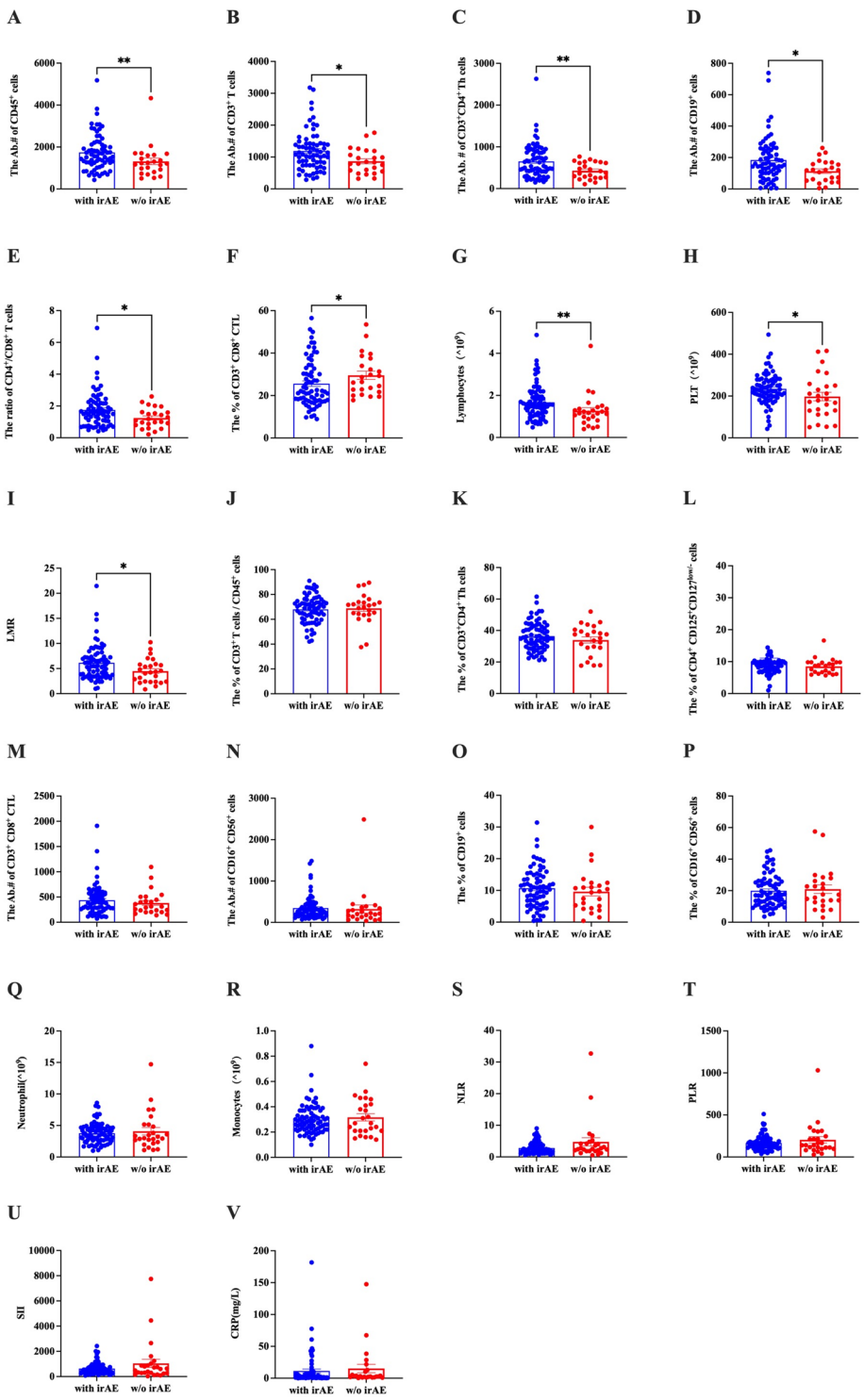
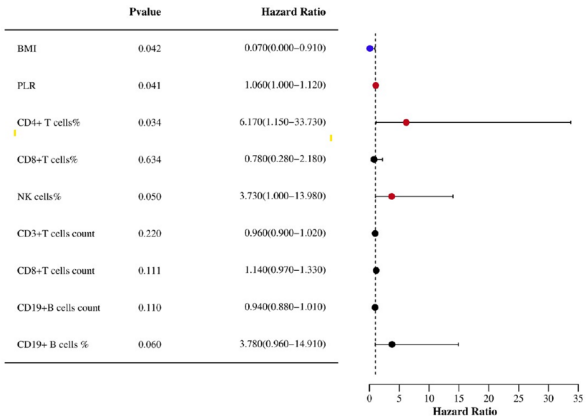
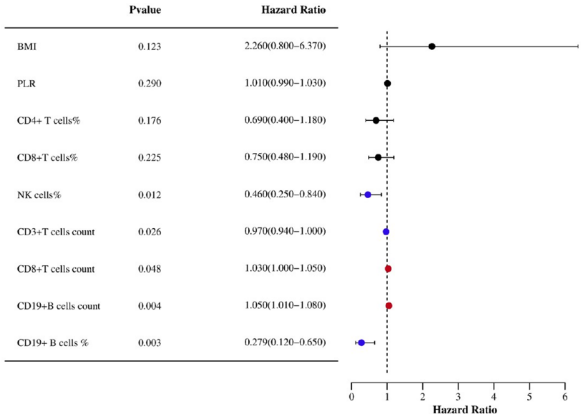


Fig. 2. Baseline clinical hematological characteristics were related to irAE onset. (**A**-**I**) The parameters of peripheral blood immunophenotypes and routine tests were analyzed in connection with irAE occurrence. (**J**-**V**) The parameters of circulating immunophenotypes and blood routine tests were irrelevant to irAE onset. Data were shown as mean ± SEM. Statistical differences between the two groups were detected by unpaired t-tests and were indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$.

A



B



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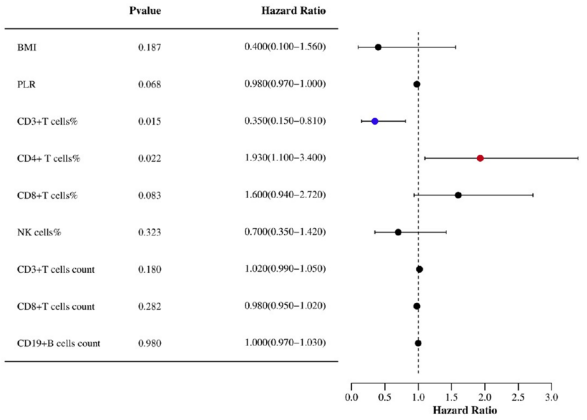

Fig. 3. Multivariate analyses demonstrated that baseline demographics, peripheral blood immunophenotypes, and hematological characteristics were correlated with treatment responses, irAEs, or prognosis. (**A**) Forest plots show the Cox regression analysis of factors related to treatment response. (**B**) Forest plots demonstrate the logistic regression analysis factors related to irAEs. (**C**) Forest plots exhibit the logistic regression analysis of factors associated with clinical outcomes. Dots and bars illustrated the hazard ratios and corresponding 95% CIs for each factor assessed. The dashed line represented the reference value of x = 1.

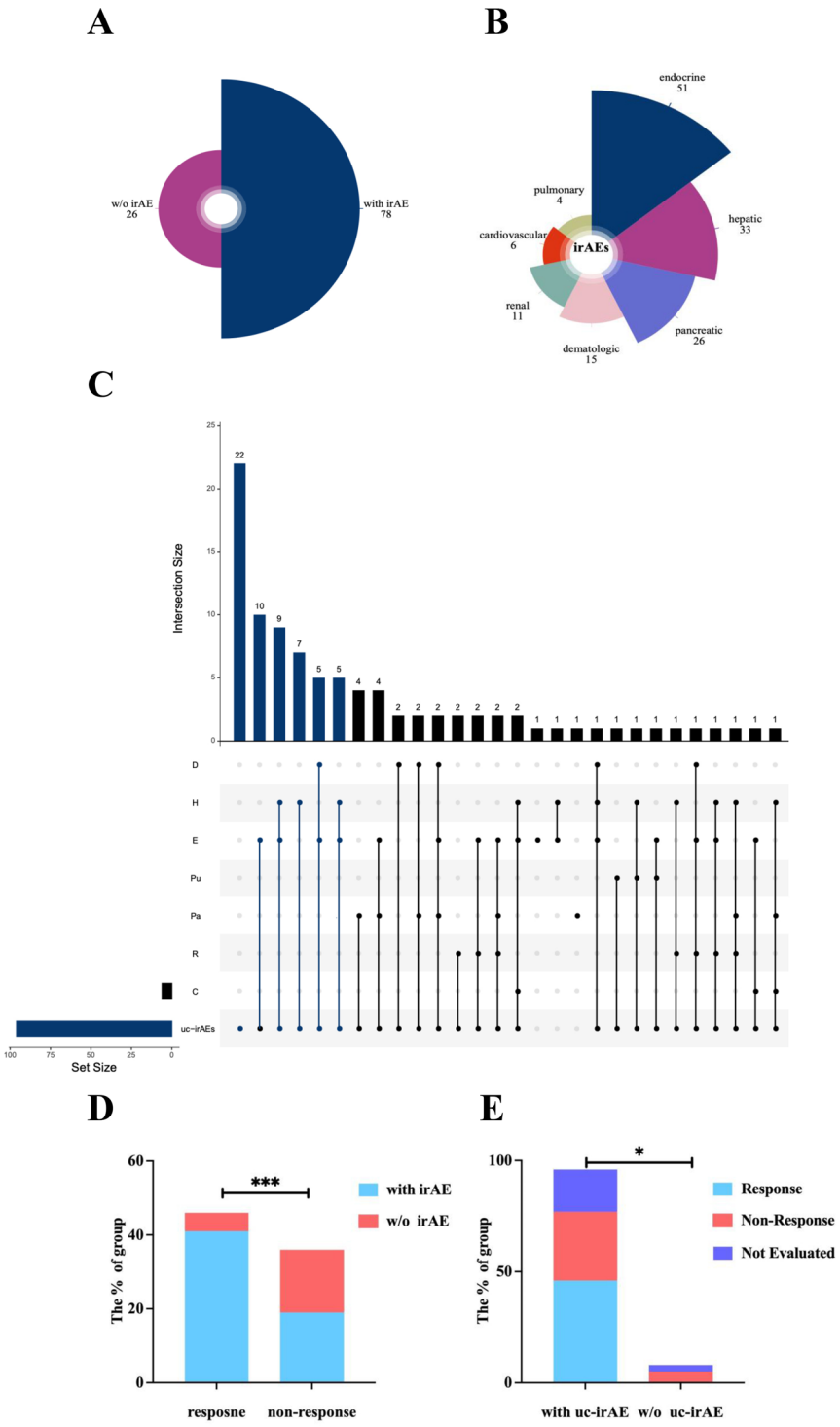

Fig. 4. The distribution of irAEs and uc-irAEs. (**A**) The distribution of patients with and without irAEs. (**B**) The distribution of irAEs according to different systems. (**C**) The overlapping pattern features between the uc-irAE and irAE groups, with single uc-irAE and the top five most frequent overlaps highlighted in dark blue. (**D**) The treatment response profiles between the with and without uc-irAE groups. The response profiles were compared using the Chi-squared test. Statistic differences were indicated as follows: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$. Abbreviations: D, dermatologic; H, hepatic; E, endocrine; Pu, pulmonary; Pa, pancreatic; R, renal; C, cardiovascular.

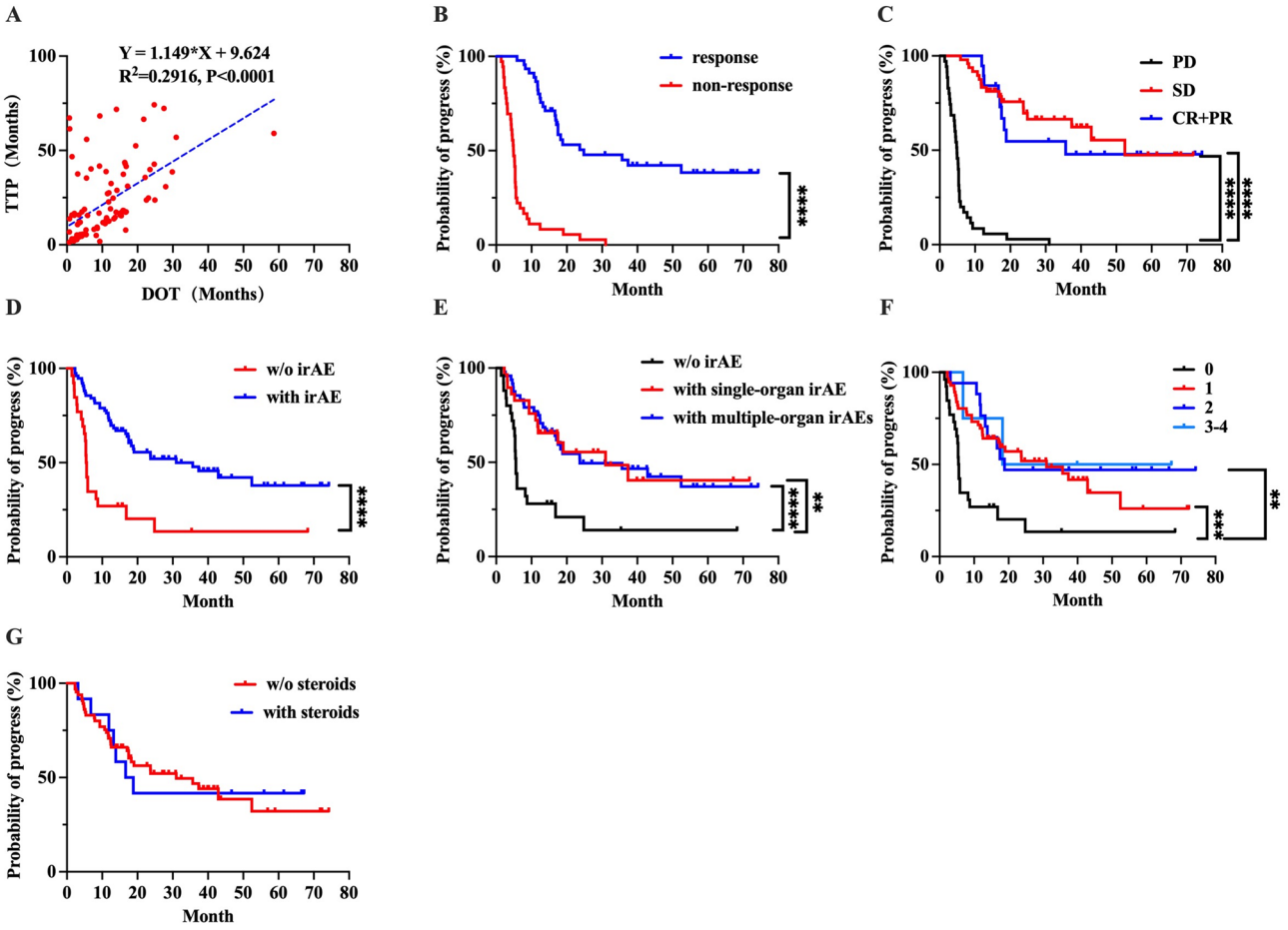

Fig. 5. Analysis of TTP among various treatment outcomes and irAEs groups. (**A**) The correlation of DOT and TTP. (**B**) The Kaplan-Meier analysis of TTP in treatment responders vs. non-responders. (**C**) KM curves of TTP in CR, PR, and SD groups. (**D**) KM curves of TTP in patients with and without irAE groups. (**E**) KM curves of TTP in groups with no irAEs, single-system irAEs, and multisystem irAEs. (**F**) KM curves of TTP across different irAE grades. (**G**) KM curves of TTP in irAE patients with and without steroid intervention. The survival curves were derived by the Kaplan-Meier method, and survival curves were compared across patient groups via the log-rank test, with significance denoted as: $*p<0.05$, $**p<0.01$, $***p<0.001$, and $****p<0.0001$.

The median TTP in patients with irAEs was significantly prolonged than those without irAEs (Figs. 5D and 31 months vs. 5.4 months, $P<0.0001$). Patients with either single- or multi-system irAEs showed a superior TTP than those without irAEs (Fig. 5E, $P<0.01$ and $P<0.0001$, respectively). While patients with single- and multi-system irAEs exhibited a similar TTP (Figs. 5E and 13.3 months vs. 12.7 months, $P>0.05$). Moreover, patients with grade 1 and grade 2 irAEs had remarkable prolonged TTP than those without irAEs (Fig. 5F, $P<0.001$ and $P<0.01$, respectively). A similar TTP was observed among patients with different grades of irAEs (Fig. 5F, $P>0.05$). Additionally, no difference was confirmed in TTP between patients with and without steroid inventions (Figs. 5G and 16.6 months vs. 31 months, $P>0.05$).

Discussion

In this study, we demonstrated that a series of baseline demographics and hematological immunophenotype characteristics were associated with anti-PD(L)-1 treatment response, irAE onset, or prognosis. A higher pretreatment percentage of $CD3^+CD4^+$ Th cells was associated with both positive responses and prognosis. The percentage of $CD16^+CD56^+$ NK cells positively related to the response but negatively associated with irAEs. Decreased $CD3^+$ T cell count was associated with more irAEs, while the decreased percentage of $CD3^+$ T cells was related to a positive prognosis. The increase in baseline $CD19^+$ B cell counts and the decrease in the percentage of $CD19^+$ B cells might correlate with more irAEs. The higher baseline PLR and $CD3^+CD8^+$ CTL cells were related to more responders or irAEs, respectively. Moreover, there was a significant correlation between DOT and TTP. IrAE onset demonstrated the dual advantage of a positive response and better outcomes than those without irAE. Notably, separately or synchronously appearing uc-irAEs may also indicate therapeutic benefits.

Consistent with previous reports, our observations have investigated a series of demographic features and blood-based biomarkers that might contribute to treatment response or irAE emergence. In this study, the

multivariate analysis results about the demographic parameters of smoking, sex, and ECOG were adjusted due to high deviation. We consider the deviation might originate from a limited sample size or other confounding biases, and these findings were still meaningful and consistent with previous evidence[13,16,25]. Prior data showed the "obesity paradox" might exist in tumor progression and treatment outcomes. The impact of overweight on the outcomes may be multifaceted and nonlinear, not only affected by sex and BMI definition but also by the tumor types, line of therapy, and ethnic heterogeneities of fat distribution[15]. In our observation, a lower level of BMI was associated with more anti-PD(L)-1 treatment responders. Different lines of treatment might affect anti-PD(L)-1 responses. Nonetheless, there seemed to be no response discrepancies among different PD(L)-1 inhibitors and tumor types. Further prospective studies are necessary to confirm the complex interactions between demographic characteristics and responses to ICIs.

The interplay between immune cells and tumor cells constitutes the overall immune status and tumor microenvironment[13]. Our previous results have confirmed that cancer patients present impaired immune functions with significantly lower levels of peripheral blood cell counts of $CD3^+$ T, $CD3^+CD4^+$ Th, $CD3^+CD8^+$ CTL, $CD19^+$ B, and $CD16^+CD56^+$ NK compared to healthy populations[26]. From a local perspective, an immune-inflamed tumor microenvironment enriched with $CD4^+$ T cells, $CD8^+$ T cells, B cells, NK cells, and dendritic cells could contribute to a better response[13]. Pretreatment circulating T cell characteristics and immune cell ratios may be associated with irAE development and severe irAEs[10,27]. We observed a clear association between a higher pretreatment percentage of $CD3^+CD4^+$ Th cells and positive response and prognosis, while the higher baseline $CD3^+CD8^+$ CTL cells were only related to more irAEs. Although more attention was focused on the potency of $CD3^+CD8^+$ CTL cells in anti-tumor immunity, the potential role of $CD3^+CD4^+$ Th cells in cancer immunotherapy needs to be explored and understood more in-depth[28]. Moreover, the biomarker associated with positive responses was not always related to more irAEs. The percentage of $CD16^+CD56^+$ NK cells was positively related to the response but negatively associated with irAEs. Additionally, circulating immune cell counts and their percentages did not necessarily play a consistent predicting role. Decreased $CD3^+$ T cell count was associated with more irAEs, while the decreased percentage of $CD3^+$ T cells was related to a favorable prognosis. Both the increase in baseline $CD19^+$ B cell counts and the decrease in the percentage of $CD19^+$ B cells might correlate with more irAEs.

An early study based on NSCLC patients treated with PD-1 inhibitors revealed that low baseline PLR was significantly associated with the occurrence of irAEs (OR 2.8, $P=0.003$). Multivariate analysis confirmed PLR as an independent predictor of irAEs (OR 2.3, $P=0.020$). Low PLR was correlated with longer PFS and OS, but not with DCR or response rate[29]. Another meta-analysis of NSCLC patients treated with PD1 inhibitors indicated that high PLR was associated with shorter OS (pooled HR 2.12, $P=0.002$) and increased risk of PFS (HR 1.61, $P<0.001$)[30]. In our observation, a high level of PLR was associated with a positive response in multivariate analysis. High PLR may originate from increased platelet and/or decreased lymphocyte counts. A drastic increase in PLR might be associated with disease progression, but the connection to ICI treatment response needs more prospective or large sample evidence. The reason is mainly because the response to ICI is more susceptible to the influence of overall immune status and tumor immune microenvironments[13]. In addition, the higher pretreatment routine blood lymphocyte counts, platelet counts, and LMR were found to be related to an increased risk of irAEs in univariate but not in multivariate analysis. Given the limited sample size and other confounding biases, more efforts were needed to confirm the above-mentioned results. However, neither the peripheral blood routine tests nor the biochemical profiles could predict response and prognosis in this study. As recent machine learning models for ICI response or large language models for irAE occurrence[31,32], more work is pushing ahead to combine clinical findings, large-scale sample sizes, and artificial intelligence to establish reliable predictive models.

Although irAEs can affect any organ, most studies have shown irAEs usually occur at barrier sites of the body, including skin, liver, lung, and gastrointestinal tract[3,33]. Our results indicated that the most prevalent any-grade irAEs were endocrine, pancreatic, and hepatic toxicities during anti-PD monotherapy. Endocrine toxicity came to the first place mainly because its profile covered hypothyroidism, hyperthyroidism, hyperglycemia, and primary adrenal insufficiency. Except for irAEs classified according to current standards, we observed a series of uc-irAEs that occurred alone or simultaneously with definite irAEs. The onset of uc-irAEs may also serve as an indicator of treatment response. Recognition of uc-irAEs might expand our knowledge of the irAE category system, help us select more potentially beneficial patients, and improve irAE monitoring and administration.

Previous reports revealed that it took more than three months for ICIs to achieve a response. Still, there was no additional survival benefit between terminating and extending therapy after two years of therapy[34,35]. Our results showed that DOT was associated with TTP mainly due to only one patient accepting anti-PD monotherapy over two years. Both previous investigations[3,8,9] and our results in this study have verified that irAE onset is related to positive response and prognosis. The mechanism underlying this relationship might be that ICIs can enhance T cell activation, promote cytokine release, increase autoimmune antibody secretion, and lead to subclinical inflammation, enhance both tumor cell killing and normal tissues being attacked, ultimately resulting in more favorable treatment response, irAE risks, and preferable prognosis[13,36,37].

In addition, the definition of response (CR + PR + SD) in this study was slightly different from the routine conception (CR + PR). In general, nearly 30% of patients with ICI therapy achieve SD as the best response, while the proportion of radiological SD response in clinical trials and translational studies was less classified as an obvious therapy benefit[38]. In our observation, SD patients exhibited non-inferiority TTP compared to CR/PR patients (23.7 months vs. 18.2 months, $P>0.05$). Recent research proposed the definition of SD responders (PFS > 6 months with no tumor growth) to distinguish those ICI-beneficial patients from non-responders[38]. Inconsistent findings also manifested in prognostic disparities among irAE-affected systems, different grades, and steroid interventions. Previous reports suggested multi-system, grade 2 or lower irAEs were associated with better outcomes than single-system, severe grade irAEs, especially for dermatological and endocrine irAEs[39,40].

Moreover, short-term steroid utilization and steroids for irAE management did not affect survival benefits from ICIs, but early administration within 2 months after ICI initiation, long-term steroid exposure, and high dose (≥ 60 mg prednisone or equivalent) approaches could lead to a dismal prognosis[41-44]. In our observation, single or multiple system irAEs, different irAE grades, and systemic steroids administration demonstrated similar clinical outcomes, with TTP showing no statistical difference. One possible explanation might be the incidence of grade 3 or higher irAEs (5%) was lower than previously reported data (10–20%)[3]. Only four patients were reported with severe irAEs, while grade 1 irAEs exceeded 70% in this study.

This study has several limitations. The results were entirely derived from a single-center retrospective study with a limited sample size. In addition, different tumor types and lines of therapy can lead to unavoidable biases. Lastly, peripheral blood analysis can only provide a unilateral perspective or partial information. Combining with tissue-based evaluation might draw more holistic and far-reaching conclusions. All the aforementioned aspects require cautious interpretation of the results and may restrict the broad application of the conclusions.

In summary, our study analyzed the roles of baseline clinical characteristics in PD(L)-1 targeted responses, irAE onset, and prognosis. Additionally, we observed single or simultaneously appearing irAEs and uc-irAEs might also be associated with treatment response, which holds potential biomarker significance, expands the scope of current irAE terms, and advances irAE administration.

Data availability

All datasets generated during and/or analyzed in this study are available from the corresponding author on reasonable request.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval
This observational study was approved by the Ethics Committee of the Affiliated Cancer Hospital of Zhengzhou University & Henan Cancer Hospital. The requirement for written informed consent was waived.

Statement & declarations
All methods were performed in accordance with the relevant guidelines and regulations.

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