

## Association between immune-related adverse event timing and treatment outcomes

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### ABSTRACT

The timing of immune-related adverse events (irAE) associated with immune checkpoint inhibitors (ICI) is highly variable. Although the development of irAE has been associated with ICI clinical benefit, how irAE timing influences this association is unknown. We analyzed two independent cohorts including 154 patients with non-small cell lung cancer (NSCLC) treated with PD-1/PD-L1 inhibitors at a single institution (UTSW cohort) and a multi-center cohort of 433 patients with NSCLC who received second-line anti-PD-1/PD-L1 therapy (Global cohort) to assess the association between ICI outcomes and irAE timing. In both cohorts, late-onset irAE occurring more than 3 months after ICI initiation compared to irAE occurring earlier were associated with greater rates of radiographic response (UTSW cohort, 41% versus 28%,  $P = .26$ ; Global cohort, 60% versus 35%,  $P = .02$ ), longer progression-free (UTSW cohort, 13.7 versus 5.6 months,  $P < .01$ ; Global cohort, not reached versus 6.0 months,  $P < .01$ ) and overall survival (UTSW cohort, 30.9 versus 14.6 months,  $P < .01$ ; Global cohort, not reached versus 10.6 months,  $P < .01$ ). Modified landmark analysis at 6 months confirmed an overall survival difference between early- and late-onset irAE. Late-onset irAE was similarly associated with greater response rates and prolonged survival in a cohort of 130 patients with non-NSCLC malignancies, suggesting a conserved association across tumor types. The favorable association between irAE and ICI clinical outcomes may be attributed to later-onset events, which is not wholly explained by survivor bias. These results allude to a distinct biology between early- and late-onset irAE and may guide clinician expectations and thresholds for continuing or modifying immunotherapy.

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## Introduction

The search for optimal predictors of immune checkpoint inhibitor (ICI) benefit continues more than a decade after these agents were first studied in clinical trials. Tumor-based biomarkers including programmed death 1 ligand (PD-L1) expression, tumor mutational burden (TMB), and microsatellite instability (MSI) are approved tests to select patients for single-agent ICI and combination ICI regimens.<sup>1–5</sup> Other exploratory biomarkers based on cancer-cell intrinsic properties include DNA repair gene mutations, immune cell exclusion, and oncogenic signaling pathways.<sup>6–9</sup> Several host factors have also been demonstrated to be putative mediators of ICI activity including HLA genotype and baseline levels of innate immune cells.<sup>10,11</sup>

In addition to these tissue- and blood-based parameters, a number of clinical characteristics have been associated with ICI efficacy. Exposure to steroids, presumably reflecting their

immunosuppressive effects, is associated with worse outcomes.<sup>12</sup> Likewise, receipt of antibiotics, which are known to alter the gut microbiome, appears detrimental to ICI benefit.<sup>13,14</sup> Smoking, particularly in lung cancer cases, is associated with increased number of tumor mutations and heightened sensitivity to ICI.<sup>2</sup> Additionally, multiple studies have shown that obese and overweight patients have superior outcomes to smaller individuals.<sup>15,16</sup>

One of the most striking observations is the link between ICI benefit and their autoimmune toxicities and immune-related adverse events (irAE). Several studies have demonstrated an association across ICI types, with some evidence that the higher the grade and the greater the number of irAE, the more pronounced the ICI clinical benefit.<sup>17–24</sup> With ICI, this association likely goes beyond pharmacokinetic considerations and also reflects cross-reactivity between anti-tumor and anti-self immune reactions. For instance, among patients with

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acute leukemia treated with allogeneic stem cell transplant, longer recurrence-free survival is associated with graft-versus-host disease.<sup>25</sup> Similarly, melanoma patients treated with cytokine therapies live longer if they develop vitiligo.<sup>26</sup>

Complicating the association between irAE and ICI benefit is the idiosyncratic nature of these toxicities. These potentially severe toxicities may affect almost any organ system and may occur at almost any point in therapy, in some cases even months after the last ICI dose.<sup>27–30</sup> In contrast, toxicities related to conventional chemotherapy and targeted therapies frequently occur in well-recognized temporal patterns that are explained by pharmacokinetics or biochemical properties of drug-target engagement. Thus, irAE occurring at different times during a patient's treatment may represent distinct biologic events. Given the heterogeneous presentation of irAE, we sought to assess whether irAE timing is associated with clinical outcomes. To account for immortal time bias – that is, the requirement that patients remain alive for a certain period of time to experience later-onset toxicities – we used time-dependent Cox regression models and landmark analyses.

## Methods

### Study setting and data collection

We assembled an exploratory cohort (UTSW cohort) composed of adult patients ( $\geq 18$  years old) who were prospectively enrolled in a registry of ICI-treated patients in the Harold C. Simmons Comprehensive Cancer Center at the University of Texas Southwestern Medical Center (UTSW) in Dallas, Texas (IRB #STU 082015–053). For primary analysis, we included patients with advanced stage non-small cell lung cancer (NSCLC) without prior ICI exposure who initiated anti-PD-1/PD-L1 therapy between November 2015 (cohort initiation) and March 2020 (to allow sufficient clinical follow-up). Given the known variability of irAE assessment among clinicians,<sup>31</sup> for each patient, irAE was characterized by the treating oncologist and two independent clinician reviewers, with no discrepancies adjudicated by a third clinician reviewer. An independent review of irAE was conducted using manual abstraction of medical records including clinic notes, telephone encounters, radiology images and reports, laboratory results, medication lists, and hospitalization records starting 1 month prior to the ICI initiation date and extending up to 3 months after the last date of ICI administration. We selected this time frame to establish baseline parameters and capture delayed-onset irAE. The last available medical oncology clinic note was also reviewed to assess the possibility of later-onset irAE and long-term sequelae. Type and grade of irAE was based on the Common Terminology Criteria for Adverse Events version 5.0. Identical procedures were used to characterize patients with cancer types other than NSCLC in the same registry. Median follow-up across the UTSW cohort was 13.0 months (interquartile range [IQR] 5.3–24.5 months). Reviewers who abstracted irAE were blinded to clinical outcomes including response, date of progression, and overall survival.

We validated initial findings from the UTSW cohort in a global multi-institutional cohort of patients with NSCLC, herein referred to as the Global cohort, which has in part

been previously described.<sup>32</sup> Briefly, the Global cohort included 433 adult patients with advanced non-small cell lung cancer (NSCLC) who received at least one dose of single-agent anti-PD-1/PD-L1 therapy in the second-line setting. The Global cohort included patient data from two institutions in the US (East Carolina University and The Ohio State University Comprehensive Cancer Center), one institution in Japan (Sendai Kousei Hospital), and two institutions in Italy (University of L'Aquila and Santa Maria della Misericordia Hospital). The study was approved by the IRB or equivalent at each site. Investigators at the respective sites adjudicated irAE assessment. Only treatment-emergent adverse events suspected to have an immunological basis as deemed by the reporting physician and the study adjudicator were categorized as irAE. Median follow-up in the Global cohort was 6.6 months (IQR 2.8–15.0 months).

Across both cohorts, we collected the following clinical data: patient age, sex, ECOG performance status, cancer type, ICI initiation and completion dates, date of clinical or radiographic disease progression (according to Response Evaluation Criteria in Solid Tumors 1.1), date of death, last date of known follow-up if no documented date of death and irAE grade and date of onset. We calculated irAE timing as the interval between the first ICI dose and the first occurrence of any irAE. Overall survival (OS) was calculated as the time from the first ICI dose to death from any cause. Progression-free survival (PFS) was calculated as the time from the first ICI dose to disease progression (clinical and/or radiographic) or death from any cause. Objective response rates were categorized as defined by Response Evaluation Criteria in Solid Tumors v1.1.

We also evaluated the association between irAE onset timing and clinical outcomes in 130 patients with other cancer types. As the Global cohort consisted of NSCLC cases only, these preliminary analyses were limited to the UTSW cohort.

### Statistical analysis

To assess the association between survival and irAE, we determined adjusted hazard ratios using Cox regression models of patients in the UTSW cohort who developed irAE adjusting for age, sex, obesity, ECOG performance status, treatment type (monotherapy, combination with chemotherapy, combination with anti-CTLA-4 antibody), line of therapy, use of steroids for irAE management, and cancer type. Since the timing of irAE onset is highly variable, we included irAE latency as a time-dependent covariate in regression models. The time-varying property of irAE latency was confirmed by analyzing Schoenfeld residuals demonstrating a violation of the proportional hazard assumption. To identify a specific cutoff time when irAE development is most likely to affect outcomes, we transformed irAE latency from a continuous covariate into an ordinal variable of one-month intervals in a time-dependent Cox regression model.

We used chi-square tests to determine the association between irAE latency and objective response rates. Median OS and PFS in patients according to irAE development and timing were calculated using the Kaplan–Meier method and the Log-rank test, respectively. To confirm that differences in Kaplan–Meier survival curves between patients who developed

**Table 1.** Characteristics of patients with NSCLC (UTSW cohort) treated with anti-PD-1/PD-L1 inhibitors.

Characteristics		No. (%) or median (IQR)
Age		68 (61–74)
Sex	Female	62 (40)
	Male	92 (60)
Race	White	128 (83)
	Black	14 (9)
	Asian	7 (5)
	Unknown	5 (3)
ECOG	0	37 (24)
	1	90 (57)
	2	27 (16)
No. of irAE	0	55 (36)
	1	30 (19)
	2	37 (24)
	>2	32 (21)
	>2	42 (27)
Highest irAE grade	1	33 (21)
	2	24 (16)
	>2	42 (27)
Treatment	ICI monotherapy	107 (70)
	Combination ICI	10 (6)
	ICI plus other therapy	37 (24)
Line of therapy	First	67 (44)
	Second	72 (47)
	Third or greater	13 (9)

early- and late-onset irAE could not be attributed to immortal time bias, we used 6-month landmark analysis to examine patients who developed irAE within 6 months of ICI initiation and remained alive after the landmark time. In these analyses, patients were stratified by irAE latency, with early-onset irAE defined as irAE occurring within 3 months of ICI initiation and late-onset irAE defined as irAE occurring between 3 and 6 months after ICI initiation. Outcome events were only considered if they occurred after the 6-month landmark. Data analysis was performed using Stata 17 (StataCorp, Texas) and SPSS 24 (IBM, New York).

## Results

Among 154 patients in the UTSW cohort, median age was 68 years and 60% were male (Table 1). One or more irAE developed in 99 patients (64%), of whom 42 (27%) developed only grade 1 toxicities and 57 (37%) developed at least one grade 2 toxicity. Most patients received single-agent ICI (68%) and received ICI in the first- or second-line setting (91%).

### Defining an irAE timing cutoff associated with prognosis

Among patients in the UTSW cohort who developed irAE, multivariable Cox regression analysis including irAE latency as a continuous time-dependent variable showed that longer time to irAE onset was significantly associated with greater PFS (HR 0.93; 95% CI, 0.87–0.99;  $P = .03$ ) and OS (HR 0.90; 95% CI, 0.83–0.98;  $P = .02$ ) after adjusting for sex, age, obesity, ECOG performance status, treatment type (single-agent or combination therapy), line of therapy, and receipt of steroids to treat toxicities (Table 2). In a time-dependent Cox regression model of patients who developed irAE including irAE latency as an ordinal variable, the development of irAE within the first 3 months after ICI initiation but not in subsequent months was associated with a greater risk of death compared to patients who developed

irAE after the fifth month of ICI treatment (Supplementary Table 1). We therefore used 3 months as a cutoff to define early-onset ( $\leq 3$  months from ICI initiation) and late-onset ( $> 3$  months from ICI initiation) irAE in subsequent analyses.

### Response rates associated with early- and late-onset irAE

In the UTSW cohort, objective response rates to ICIs were greater in patients with late-onset irAE (41%) than in patients with early-onset irAE (28%), although the difference was not statistically significant ( $P = .26$ ). However, compared to patients with no irAE, the odds ratio (OR) of an objective response was greater and more strongly associated with patients with late-onset irAE (OR 3.6,  $P = .002$ ) than patients with early-onset irAE (OR 2.1,  $P = .11$ ) (Figure 1). Notably, the median time to response was equivalent between patients with no irAE (2.7 months, interquartile range 1.8–3.3 months), early-onset irAE (2.5 months, interquartile range 1.7–3.7 months), and late-onset irAE (2.6 months, interquartile range 1.6–3.6 months) (Supplementary Figure 1). Similar analyses of the Global cohort showed that the radiographic response rate in patients with early-onset irAE was greater than in patients without irAE (35% versus 10%,  $P < .001$ ) (Figure 1). Moreover, the radiographic response rate in patients with late-onset irAE was significantly greater than patients with early-onset irAE (60% versus 35%,  $P = .02$ ) (Figure 1).

### Progression-free and overall survival associated with early- and late-onset irAE

In the UTSW cohort, the median PFS for patients with no irAE, early-onset irAE, and late-onset irAE was 2.8, 5.6, and 13.8 months, respectively (Log-rank test,  $P < .01$ ) (Figure 2). The median OS for patients with no irAE, early-onset irAE, and late-onset irAE was 9.1, 14.2, and 30.9 months, respectively ( $P < .01$ ) (Figure 2). In the Global cohort, the median PFS for patients with no irAE, early-onset irAE, and late-onset irAE was 2.3 months, 6.0 months, and not reached, respectively ( $P < .01$ ) (Figure 2). The median OS for patients with no irAE, early-onset irAE, and late-onset irAE was 4.7 months, 10.6 months, and not reached, respectively ( $P < .01$ ), respectively (Figure 2). Using a nominal variable to categorize patients with no irAE, early-onset irAE, or late-onset irAE in multivariable Cox regression analyses showed that patients with late-onset irAE had longer OS and PFS than patients with no irAE or early-onset irAE even after adjusting for other clinical factors (Supplementary Table 2 and Supplementary Table 3).

### Modified 6-month landmark analysis of patients who developed irAE

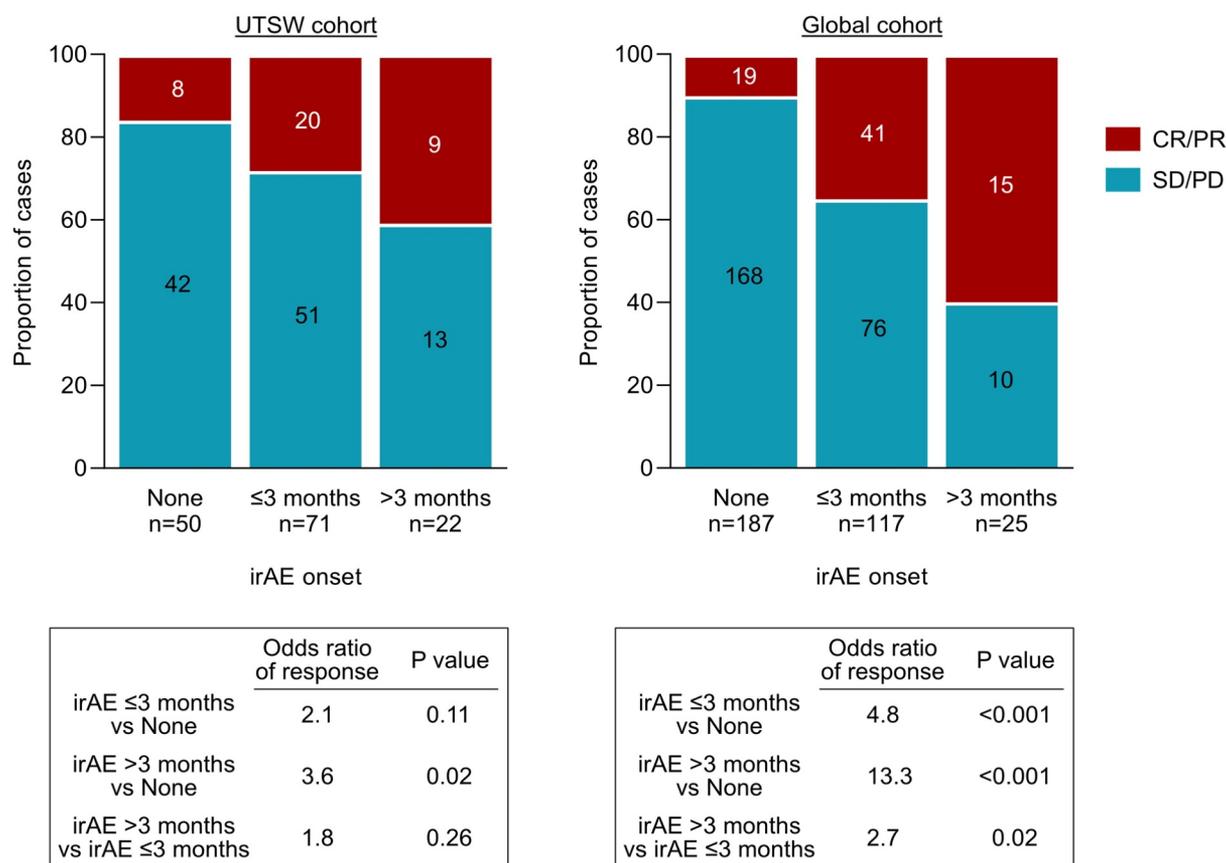
To confirm that the difference in survival between patients with early- and late-onset irAE is not entirely explained by early deaths among patients with early-onset irAE, we used a modified 6-month landmark analysis of patients who developed irAE in the UTSW and Global cohorts. In this

**Table 2.** Multivariable Cox regression models for progression-free and overall survival including irAE latency as a time-dependent variable in patients treated with PD-1/PD-L1 inhibitors who developed irAE in the UTSW cohort.

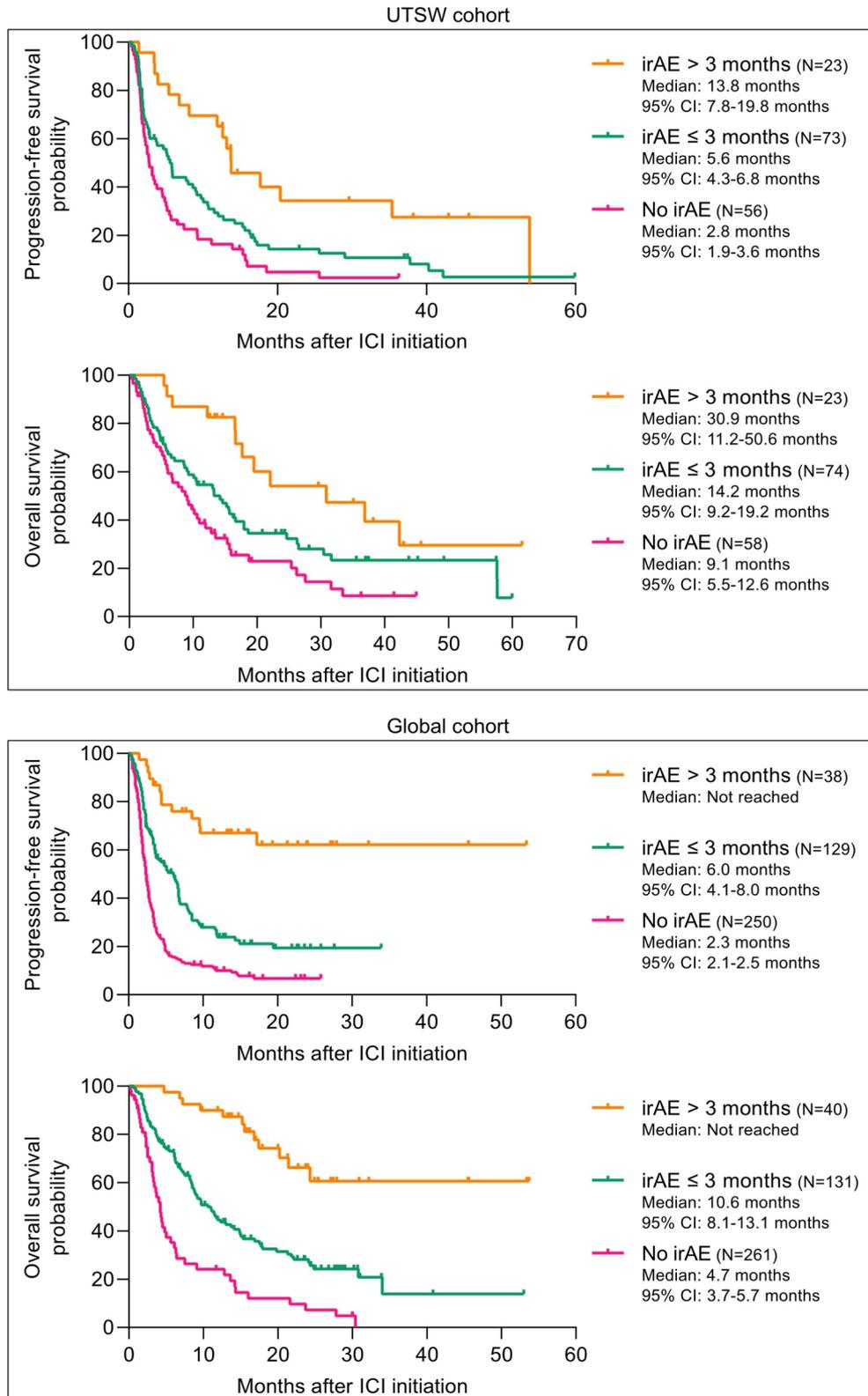
Patient characteristics		Progression-free survival		Overall survival	
		HR (95% CI)	P value	HR (95% CI)	P value
Sex	Female	Reference		Reference	
	Male	1.28 (0.73–2.26)	0.39	1.70 (0.90–3.20)	0.10
Age		1.01 (0.98–1.04)	0.60	1.00 (0.97–1.03)	0.87
Body mass index	<30	Reference		Reference	
	≥30	1.01 (0.56–1.82)	0.98	1.06 (0.56–2.01)	0.86
ECOG	0	Reference		Reference	
	1	1.64 (0.90–3.01)	0.11	2.35 (1.18–4.67)	0.02
	2	2.78 (1.21–6.39)	0.01	3.04 (1.22–7.57)	0.02
Treatment type	ICI monotherapy	Reference		Reference	
	ICI plus other therapy	1.81 (0.88–3.75)	0.11	1.33 (0.66–2.68)	0.43
	Combination ICI	0.75 (0.28–2.01)	0.56	0.77 (0.29–2.06)	0.61
Line of therapy	First	Reference		Reference	
	Second	1.42 (0.75–2.68)	0.28	1.05 (0.55–1.98)	0.89
	Third or greater	4.13 (1.48–11.53)	0.01	0.97 (0.29–3.21)	0.96
Time to irAE (months)		0.93 (0.87–0.99)	0.03	0.90 (0.83–0.98)	0.02
Steroid use for irAE	No	Reference		Reference	
	Yes	0.80 (0.49–1.31)	0.38	1.13 (0.66–1.93)	0.66

analysis, patients who died prior to 6 months or developed irAE after 6 months were excluded. Thus, while the definition of early-onset irAE remained unchanged in the 6-month landmark analyses, late-onset irAE only include events occurring between 3 and 6 months after ICI-initiation. This analysis reduces sample size and may introduce additional sources of bias, but alleviates the impact of

immortal time bias. In both the UTSW and Global cohort, there was a near-significant trend association with greater OS among patients with late-onset irAE compared to patients with early-onset irAE by the 6-month landmark time (UTSW cohort, HR 0.55, 95% CI, 0.25–1.24;  $P = .15$ ; Global cohort, HR 0.49, 95% CI, 0.26–0.91;  $P = .06$ ) (Supplementary Figure 2).



**Figure 1.** Radiographic response rates to PD-1/PD-L1 inhibitors in patients with no irAE, early-onset irAE, and late-onset irAE. Objective responses were determined by RECIST. Numbers within columns indicate the number of patients in each response category. Odds ratio of response and  $P$  values for multiple comparisons are provided below each plot, respectively. CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease. RECIST, response evaluation criteria in solid tumors.



**Figure 2.** Progression-free survival and overall survival of patients treated with no irAE, early-onset irAE, and late-onset irAE. Kaplan–Meier curves of progression-free and overall survival of patients with no irAE, irAE within 3 months of therapy initiation, and irAE after 3 months of therapy initiation are plotted for the UTSW and Global cohort. irAE, immune-related adverse event.

## Association between late-onset irAE and clinical benefit in other cancer types

To assess whether late-onset irAE is associated with clinical benefit among patients with other malignancies, we also examined additional 130 ICI-treated patients in the prospective registry at UTSW with non-NSCLC cancer types. Among patients who developed irAE, multivariable Cox regression analysis showed that longer time to irAE was associated with greater OS (HR 0.73; 95% CI, 0.54–0.98;  $P = .04$ ) after adjusting for clinical factors and cancer type (Supplementary Table 4). Compared to patients with no irAE, the likelihood of an objective response was greater among patients with late-onset irAE (OR 3.6,  $P = .05$ ) than among patients with early-onset irAE (OR 2.1,  $P = .19$ ) (Supplementary Figure 3A). The median OS for patients with no irAE, early-onset irAE, and late-onset irAE was 9.2, 34.4, and 40.0 months, respectively ( $P = .003$ ) (Supplementary Figure 3B).

## Discussion

Several studies have demonstrated an association between irAE and ICI benefits across cancer populations.<sup>17,19,21,22</sup> To our knowledge, this is the first analysis to examine the effect of irAE timing on this association. Consistent with earlier studies, we found that patients who develop irAE have more favorable outcomes. Notably, our study also demonstrated that late-onset (>3 months) irAE appears to drive this benefit, as earlier toxicities have statistically and clinically significant worse outcomes. We observed this relationship in a single-center exploratory cohort as well as a multi-center international validation cohort. We also noted these findings across efficacy parameters, including radiographic response, PFS, and OS. Furthermore, late-onset irAE remained associated with superior outcomes after controlling for multiple clinical variables. While much of this study was focused on NSCLC, our preliminary analysis of patients with other malignancies suggests that late-onset irAE may be associated with improved outcomes across multiple cancer types.

A potential confounding factor is that patients with late-onset irAE may have the best outcomes simply because they must live long enough to develop them. We accounted for this bias, termed immortal time bias, using established analytical techniques including time-dependent regression models and landmark analyses. Additionally, immortal time bias is unlikely to explain the association between irAE latency and radiographic response rates because the time to response was equivalent between patients without irAE, with early-onset irAE, and with late-onset irAE.

The mechanisms of irAE remain to be fully characterized, though early evidence indicates that genetic variants associated with autoimmune skin disorders are also associated with ICI outcomes and skin irAE, suggesting overlapping mechanisms of efficacy and toxicity.<sup>35</sup> In support of this, shared T-cell antigens between tumor tissue and irAE-related skin lesions have been reported in patients with NSCLC who responded to ICI.<sup>36</sup> Our results suggest that early- and late-onset irAE may

have differing underlying biology. It is noteworthy that the time cutoff of 100 days that distinguishes acute from chronic graft-versus-host disease (GVHD) is similar to our cutoff distinguishing early- and late-onset irAE.<sup>37</sup> While the pathophysiology of acute and chronic GVHD are overlapping, immune cell profiling studies indicate that chronic GVHD is associated with altered immune cell populations including decreased regulatory NK- and B-cells.<sup>34</sup> The role of a dysregulated humoral immune response in late-onset irAE is supported by an association between increased autoantibody production prior to the development of a Raynaud-like phenomenon in a patient nearly 20 months after ICI initiation, which was not observed after the development of an earlier-onset irAE in the same patient.<sup>38</sup> Other studies have similarly implicated autoantibodies and preexisting humoral autoimmune diseases as risk factors for irAE, but whether distinct immune mechanisms are responsible for early- and late-onset irAE remains to be investigated.<sup>38–40</sup> In addition, whether the effectiveness of steroids or other immunosuppressive agents in early- and late-onset irAE differs is unknown.

Strengths of this study include the rigorous process of irAE data abstraction, the interrogation of two independent datasets, the inclusion of a radiographic response endpoint, and the use of established statistical approaches to account for potential biases. Limitations include the retrospective nature of the study, the lack of biospecimens to conduct correlative studies, limited data on other medications that may have been taken during ICI treatment, a limited sample size of non-lung cancer types, and the short median follow-up in the Global cohort. While our results were based on cohorts from multiple institutions, the generalizability of findings remains to be clarified. From this study it is also not possible to determine the optimal time-point for defining late-onset irAE. Indeed, prior analyses using a 12-month cutoff to define early- and late-onset irAE did not show a difference in survival, suggesting that there is a lower and upper bound to defining irAE associated with clinical benefit.<sup>33</sup>

In conclusion, even when controlling for multiple clinical variables, examining multiple efficacy endpoints, and accounting for immortal time bias, we find that irAE timing has a major effect on the association between ICI toxicity and efficacy. We note that this work is hypothesis generating, as the biological basis for the differing outcomes between early-onset and late-onset irAE remains to be elucidated, and future studies are needed to clarify the temporal relationship between anti-tumor and anti-host effects of ICI. In addition, the contribution of organ-specific irAEs to the clinical benefit of ICIs remains to be investigated as the limited incidence of specific types of irAEs precluded a rigorous analysis in this study. Nonetheless, our observations may ultimately inform clinicians' expectations, communications, and thresholds for continuing or modifying therapy.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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## Statement of Translational Relevance

Immune related-adverse events (irAE) vary widely in their time to onset and have been associated with improved outcomes from immune checkpoint inhibitors (ICI). In this study, we demonstrate that irAE timing is a critical determinant of the association with ICI benefit. Specifically, irAE developing later than 3 months after ICI initiation is associated with greater objective response rates and survival. These results suggest distinct tumor or host biology underlying late-onset irAE and may guide clinician expectations and thresholds for continuing or modifying immunotherapy. Our findings warrant further investigation to identify biologic explanations and potential biomarkers which may be used to anticipate ICI toxicities and benefits.

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