ONCOIMMUNOLOGY
2024, VOL. 13, NO. 1, 2351255
https://doi.org/10.1080/2162402X.2024.2351255

Taylor & Francis
Taylor & Francis Group

BRIEF REPORT

Distinct autoantibody profiles across checkpoint inhibitor types and toxicities

Hong Mu-Mosley[a], Mitchell S. von Itzstein[a,b], Farjana Fattah[a], Jialiang Liu[c], Chengsong Zhu[d], Yang Xie[a,c,e], Edward K. Wakeland[d], Jason Y. Park[f], Brad S. Kahl[g], Catherine S. Diefenbach[h]*, and David E. Gerber[a,b,e]*

[a]Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, TX, USA; [b]Division of Hematology-Oncology, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, TX, USA; [c]Quantitative Biomedical Research Center, UT Southwestern Medical Center, Dallas, TX, USA; [d]Department of Immunology, UT Southwestern Medical Center, Dallas, TX, USA; [e]Peter O'Donnell Jr. School of Public Health, UT Southwestern Medical Center, Dallas, TX, USA; [f]Department of Pathology, UT Southwestern Medical Center, Dallas, TX, USA; [g]School of Medicine, Washington University,Louis, MO, USA; [h]Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA

ABSTRACT

Immune checkpoint inhibitors (ICI) are increasingly used in combination. To understand the effects of different ICI categories, we characterized changes in circulating autoantibodies in patients enrolled in the E4412 trial (NCT01896999) of brentuximab vedotin (BV) plus ipilimumab, BV plus nivolumab, or BV plus ipilimumab-nivolumab for Hodgkin Lymphoma. Cycle 2 Day 1 (C2D1) autoantibody levels were compared to pre-treatment baseline. Across 112 autoantibodies tested, we generally observed increases in ipilimumab-containing regimens, with decreases noted in the nivolumab arm. Among 15 autoantibodies with significant changes at C2D1, all nivolumab cases exhibited decreases, with more than 90% of ipilimumab-exposed cases showing increases. Autoantibody profiles also showed differences according to immune-related adverse event (irAE) type, with rash generally featuring increases and liver toxicity demonstrating decreases. We conclude that dynamic autoantibody profiles may differ according to ICI category and irAE type. These findings may have relevance to clinical monitoring and irAE treatment.

ARTICLE HISTORY
Received 3 January 2024
Revised 8 April 2024
Accepted 30 April 2024

KEYWORDS
Autoantibodies; biomarkers; immune checkpoint inhibitor; PD-1; CTLA-4; immune-related adverse events; immunotherapy

Introduction

In recent years, immune checkpoint inhibitor (ICI) indications and regimens have expanded profoundly. Whereas these cancer treatments were once administered exclusively as monotherapy for previously treated, advanced malignancies, they are now approved across disease stages. Furthermore, combinations with molecularly targeted therapies, conventional chemotherapy, radiation therapy, or other ICI are approved or under investigation in multiple cancer types.

Combination immunotherapy has brought both disappointment and success. Treatment with the anti-cytotoxic T lymphocyte antigen 4 (CTLA4) antibody ipilimumab plus the BRAF inhibitor vemurafenib resulted in frequent hepatotoxicity.[1] When added to the epidermal growth factor receptor osimertinib, the anti-programmed death ligand 1 (PDL1) agent durvalumab led to unacceptable rates of pulmonary toxicity.[2] Conversely, the addition of pembrolizumab to platinum-doublet chemotherapy is generally well tolerated and effective in most patients.[3]

Among immunotherapy regimens, combination CTLA4 and PD1 (or PDL1) inhibition has produced the greatest efficacy, as well as the greatest toxicity. In metastatic melanoma, ipilimumab plus nivolumab yields response rates exceeding 90% and median survival beyond 3 years. However, these patients also face considerably higher rates and severity of immune-related adverse events (irAE) than do patients treated with either agent alone.

Autoantibodies may provide insight into the effect of ICI on host immune function. Employed as biomarkers for some autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematous,[4] they have been studied to a limited degree in the context of cancer immunotherapy. Certain antibodies have been associated with irAE risk.[5–7] Post-treatment changes in autoantibodies have also been linked with clinical outcomes.[8]

Because different types of ICI act on different components of anti-tumor immunity and are associated with different toxicity patterns, we studied dynamic autoantibody changes and irAE in a population treated with various ICI regimens.

Materials and methods

Patient population and study procedures

This study included patients with relapsed or refractory Hodgkin lymphoma treated on the Eastern Cooperative Oncology Group-American College of Radiology (ECOG-ACRIN) E4412 phase 1/2 trial of brentuximab vedotin (anti-CD30), ipilimumab (anti-CTLA4), and nivolumab (anti-PD1) (NCT01896999).[9] Key exclusion criteria included prior ICI treatment, active autoimmune disease, or significant organ

CONTACT David E. Gerber david.gerber@utsouthwestern.edu Division of Hematology-Oncology, Harold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 8852

*Co-senior authors.

Supplemental data for this article can be accessed online at https://doi.org/10.1080/2162402X.2024.2351255.

dysfunction. The E4412 trial, which was performed according to the Declaration of Helsinki and International Harmonization Guidelines for Good Practice, was approved by all participating centers. All enrolled patients provided written informed consent prior to undergoing any study-related procedures.

During a 3 + 3 dose escalation phase 1 component, patients were enrolled sequentially into cohorts receiving (a) BV 1.8 mg/kg IV plus ipilimumab 1–3 mg/kg IV (ipilimumab group), (b) BV 1.2–1.8 mg/kg IV plus nivolumab 3 mg/kg IV (nivolumab group), or BV 1.2–1.8 mg/kg IV plus ipilimumab 1 mg/kg IV plus nivolumab 3 mg/kg IV (ipilimumab + nivolumab therapy group). BV and nivolumab were given every 3 weeks; ipilimumab was administered every 6 weeks in the ipilimumab group and every 12 weeks in the ipilimumab + nivolumab group. BV was continued for up to one year (16 doses); nivolumab, up to two years (34 doses). Ipilimumab was administered for up to 1 year (7 doses) in the ipilimumab group and 2 years (9 doses) in the ipilimumab + nivolumab group.[9]

Clinical data

We obtained clinical data (including demographics, treatment assignment, and toxicities) from the E4412 study database. In the trial, toxicity assessment and laboratory evaluations were performed weekly during cycle 1 and at the start of subsequent cycles. The highest grade of each toxicity type was reported for individual patients. Adverse events were graded according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. For the present analysis, we characterized adverse events as irAE if (1) they represented recognized irAE types and (2) they were unlikely to be caused by brentuximab vedotin.

Because any grade irAE occurred in almost all patients in this study, we focused on clinically significant and higher-grade (grade ≥2 and grade ≥ 3) irAE when we examined autoantibody associations across all irAE. Due to smaller case numbers, in our analyses of individual irAE types we included all-grade irAE, designating high-grade irAE (grade ≥3) subsets within these groups. Due to the small number of patients, variety of Hodgkin's subtypes and stages, various prior types of therapy, and different treatments included in the E4412 trial, we did not include analyses of therapeutic efficacy in this study.

Specimen processing and serum autoantibody profiling

We analyzed blood samples that had been prospectively collected for correlative studies at pre-treatment baseline (within 72 h of treatment initiation) and on cycle 2 day 1 (C2D1, 21 days after treatment initiation). Samples were centrifuged and sera stored at −80°C. For the present analysis, we received aliquots from storage facilities at Mayo Clinic and MD Anderson Cancer Center.

We used autoantigen microarrays for autoantibody profiling as described previously.[10,11] Briefly, a panel of 112 autoantibodies was selected to represent autoantibodies associated with various immune-related diseases or allergic disorders (Supplemental Table S1).[11] Eight additional proteins (Ig control 1:2, Ig control 1:4, Ig control 1:8, Ig control 1:16, anti-Ig control 1:2, anti-Ig control 1:4, anti-Ig control 1:8, anti-Ig control 1:16) were imprinted on the arrays as internal controls and for data normalization.

Serum samples were first treated with DNAse I to remove free DNA and then applied onto autoantigen arrays with 1:50 dilution. Autoantibody binding was detected with cy3-labeled anti-human IgG. Array slides were scanned with a Genepix 4400A scanner with laser wavelengths 532 nm for cy3 to generate Tiff images. We used Genepix Pro 7.0 software to analyze images and to generate genepix report (GPR) files (Molecular Devices, Sunnyvale, California, USA). The net fluorescent intensity (NFI) of each antigen was generated by subtracting the local background and negative control (phosphate buffered saline) signals. We generated a signal-to-noise ratio (SNR, background) for each antigen as follows: (foreground median – background median)/standard deviation.

We used SNR as a quantitative measure of the ability to resolve true signal from background noise. A higher SNR indicates higher signal over background noise. Autoantibodies with SNR < 3 in more than 90% of the samples were excluded from further analysis.[10,11] NFI was normalized by robust linear model using positive controls with different dilutions. Log2 of normalized NFI was used for data analysis.

We calculated log fold-change (FC) between pre-treatment baseline and C2D1 for each autoantibody. For a given patient, if either the pre-treatment or C2D1 value was 0, we incremented both values by 1 before calculating the corresponding FC. We generated heatmaps with hierarchical clustering with average linkage of WPGMA using Genesis cluster analysis of microarray data.

Statistical analysis

Participant characteristics were described with medians (interquartile range) for continuous variables and frequencies (proportions) for categorical variables by treatment groups. The association of demographic and irAE characteristics with treatment groups were further assessed using Fisher's exact test for categorical variables and Kruskal-Wallis test for continuous variables. Additionally, we used Mann-Whitney U tests to compare autoantibody profiles between irAE categories and Kruskal-Wallis test among treatment groups. Two-sided p values < 0.05 were considered statistically significant. Because this was a hypothesis-generating pilot study with a relatively small sample size, we did not correct for multiple comparisons using adjusted p values or false discovery rate for statistical analysis.[12] All computation was performed with R (v4.2.2).

Results

Patient characteristics and irAE

Among a total of 64 patients enrolled in the E4412 trial, 48 patients (75%) had available baseline serum samples and were included in this study. Among these cases, 43 (90%) also had C2D1 samples and were included in analyses of autoantibody changes. Table 1 summarizes patient characteristics and irAE according to the treatment group. Mean age was 36 years, 46% were women, and 86% were White.

Table 1. Patient characteristics and immune-related adverse events by treatment group.

			Treatment			
		Total n (%)	Ipilimumab plus BV n (%)	Nivolumab plus BV n (%)	Ipilimumab + nivolumab plus BV n (%)	*P* value
N		48	19	13	16	
Age (years)						
	Median (IQR)	33.5 (16.0)	32.0 (8.5)	46.0 (25.0)	31.0 (13.0)	0.3
	Mean (SD)	35.5 (11.7)	34.4 (8.9)	41.6 (15.8)	31.9 (9.3)	0.1
Sex						
	Female	22 (46)	9 (47)	9 (69)	8 (50)	0.4
	Male	26 (54)	10 (53)	4 (31)	8 (50)	
Race*						
	White	38 (86)	17 (90)	10 (83)	11 (85)	1
	Black or African American	3 (7)	1 (5)	1 (8)	1 (8)	
	Asian	3 (7)	1 (5)	1 (8)	1 (8)	
Modified Ann Arbor Stage						
	I-II	24 (50)	9 (47)	5 (39)	10 (62)	0.6
	III	12 (25)	6 (32)	3 (23)	3 (19)	
	IV	12 (25)	4 (21)	5 (38)	3 (19)	
Hodgkin lymphoma by WHO histology*						
	302-Nodular sclerosis	39 (83)	15 (83)	9 (69)	15 (94)	0.5
	303-Lymphocyte-rich	2 (4)	1 (6)	1 (8)	0 (0)	
	304-Mixed cellularity	6 (13)	2 (11)	3 (23)	1 (6)	
Disease type						
	Refractory	13 (27)	4 (21)	5 (38)	4 (25)	0.6
	Relapsed	35 (73)	15 (79)	8 (62)	12 (75)	
Prior Brentuximab						
	Yes	6 (13)	3 (16)	3 (23)	0 (0)	0.1
	No	42 (87)	16 (84)	10 (77)	16 (100)	
Prior transplant type						
	Allogeneic	3 (6)	1 (5)	1 (8)	1 (6)	0.9
	Autologous	16 (33)	7 (37)	5 (38)	4 (25)	
	None	29 (60)	11 (58)	7 (54)	11 (69)	
Prior chemotherapy regimens						
	Median (IQR)	2 (2)	2 (2.5)	2 (2.0)	2 (1.3)	0.7
	Mean (SD)	2.2 (1.5)	2.6 (2.0)	2.0 (1.1)	1.9 (0.8)	0.3
Immune-related adverse events (irAE)						
	Grade					
	No irAE	2 (4)	0 (0)	2 (15)	0 (0)	0.3
	Mild irAE (grade ≤2)	33 (69)	14 (74)	9 (69)	10 (62)	
	Severe irAE (grade ≥3)	13 (27)	5 (26)	2 (15)	6 (38)	
	Type					
	Rash	13 (27)	7 (37)	1 (8)	5 (31)	0.07
	Diarrhea	10 (21)	6 (31)	2 (15)	2 (13)	
	Liver	8 (17)	3 (16)	4 (31)	1 (6)	
	Other	15 (31)	3 (16)	4 (31)	8 (50)	
	No irAE	2 (4)	0 (0)	2 (15)	0 (0)	

*Numbers may not sum to the total number of patients due to missing data.

Treatment assignment was as follows according to ICI type: ipilimumab ($n = 19$), nivolumab ($n = 13$), and ipilimumab + nivolumab therapy ($n = 16$).

Overall, 46 patients (96%) experienced any grade irAE, with 13 (27%) having grade ≥ 3 irAE. Rash was the most common irAE (27%), followed by diarrhea (21%), and elevated hepatic enzymes (17%). Despite the relatively low number of cases per treatment group, we observed a nearly significant difference in irAE types according to ICI type ($p = 0.07$). In general, rash and diarrhea occurred more frequently with ipilimumab-containing regimens (including combination with nivolumab), while elevated hepatic enzymes were more common in the nivolumab group. This difference was most notable for rash, which developed in 37% of ipilimumab patients, 8% of nivolumab patients, and 31% of ipilimumab + nivolumab therapy patients.

Autoantibody profiles according to treatment group

Baseline and Cycle 2 Day 1 blood samples were available for 18 ipilimumab, 11 nivolumab, and 14 ipilimumab + nivolumab cases. Across all treatment groups, we noted significant changes in levels [log2NFI(C2D1/BL)] of 15 autoantibodies (13%) (Figure 1). Among these, 7 (47%) were anti-matrix and 3 (20%) were anti-nuclear antigen. Notably, autoantibody changes clearly differed according to the treatment group. For all 15 autoantibodies with significant changes after treatment initiation, levels decreased in the nivolumab group. Conversely, all 15 autoantibodies increased in the ipilimumab plus nivolumab group, and 14 autoantibodies (93%) increased in the ipilimumab group. The greatest changes (whether decreased or increased) were observed for anti-matrix autoantibodies. Figure 2 displays changes in levels of all 112 tested autoantibodies. Similar to the subset of autoantibodies with

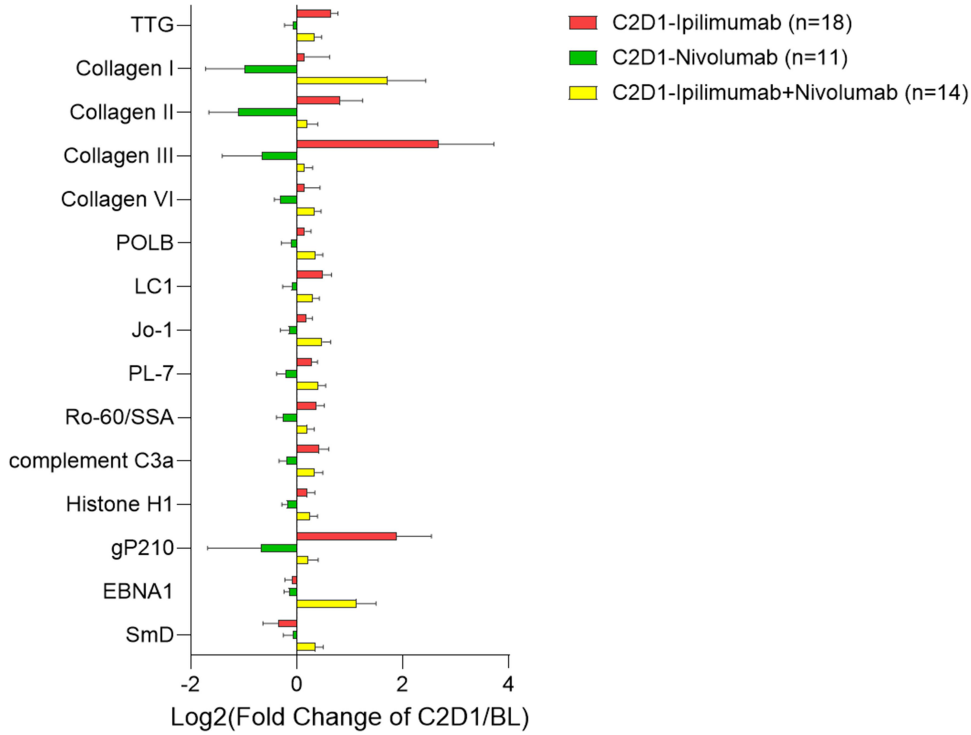

Figure 1. Autoantibodies with significant changes after ICI initiation. Fold change of log2NFI(C2D1/BL) is shown as mean ± SE. $^{**}p < 0.01$, $^{*}p < 0.05$ by Kruskal-Wallis test.

significant changes, in this larger analysis Increased levels after ICI initiation were observed most commonly with ipilimumab-containing regimens, with decreases most common in the nivolumab group.

To evaluate longitudinal trends, we analyzed autoantibody changes at a later time-point (Cycle 4 Day 1) (Supplemental Table S2). At this time-point (which occurred around Day 63), samples were available for 15/18 (83%) ipilimumab cases, 11/11 (100%) nivolumab cases, and 11/14 (79%) of ipilimumab + nivolumab cases included in the Cycle 2 Day 1 analysis. Among the 19 autoantibodies with significant differences according to ICI regimen at the early time-point ($n = 15$), the later time-point ($n = 7$), or both time-points ($n = 3$), 14 (74%) displayed the same relative changes at both time-points, suggesting that early observations may be preserved throughout ICI therapy.

Autoantibody profile associations with irAE

Supplemental Figure S1 shows autoantibody changes according to specific irAE type, grade, and ICI regimen. Increases in autoantibody levels were most prominent for rash, while liver toxicity (autoimmune hepatitis) generally featured decreased levels. Interestingly, for rash cases, autoantibody increases appeared to be greatest in lower-grade (grade ≤2) irAE. Because rash (particularly high-grade cases) occurred at greater frequency than might be expected from ICI regimens, we identified those antibodies with significantly different changes in rash cases compared to other irAE ($n = 32$) (Supplemental Figure S2). Supplemental Figure S3 highlights those autoantibodies with significant changes (almost all of which were decreases) in liver irAE.

We also compared the specific autoantibody changes observed in association with ICI regimen and those observed in association with rash and liver irAE (Supplemental Table S3). Among autoantibody changes significantly associated with ICI regimen ($n = 15$), rash irAE ($n = 32$), and liver irAE ($n = 18$), 9 were shared between ICI regimen and rash irAE, and only one was shared between ICI regimen and liver irAE.

Discussion

Given the growing use and complexity of combining ICI regimens for the treatment of various cancers, we analyzed autoantibodies and irAE in a cohort of patients with Hodgkin lymphoma treated with multi-agent regimens incorporating anti-CD30, anti-PD1, and anti-CTLA4 treatments. Although the sample size in this study was relatively small, to our knowledge this report represents one of the first to examine differences in dynamic systemic immune parameters according to ICI category. Earlier autoantibody analyses in ICI populations have either focused exclusively on pre-treatment baseline metrics or have included only a single type of ICI regimen.[7,13–15]

We observed clear differences in autoantibody changes by ICI type. In general, anti-PD1 therapy was associated with decreases in autoantibody profiles after one cycle of treatment. Anti-CTLA-4-based regimens – with or without concomitant PD1 therapy – tended to have increases in autoantibody profiles. These patterns persisted over time. The expected biological effects of these treatments may support these findings. CTLA-4 expression inhibits CD8+ CD28+ T-cell functions

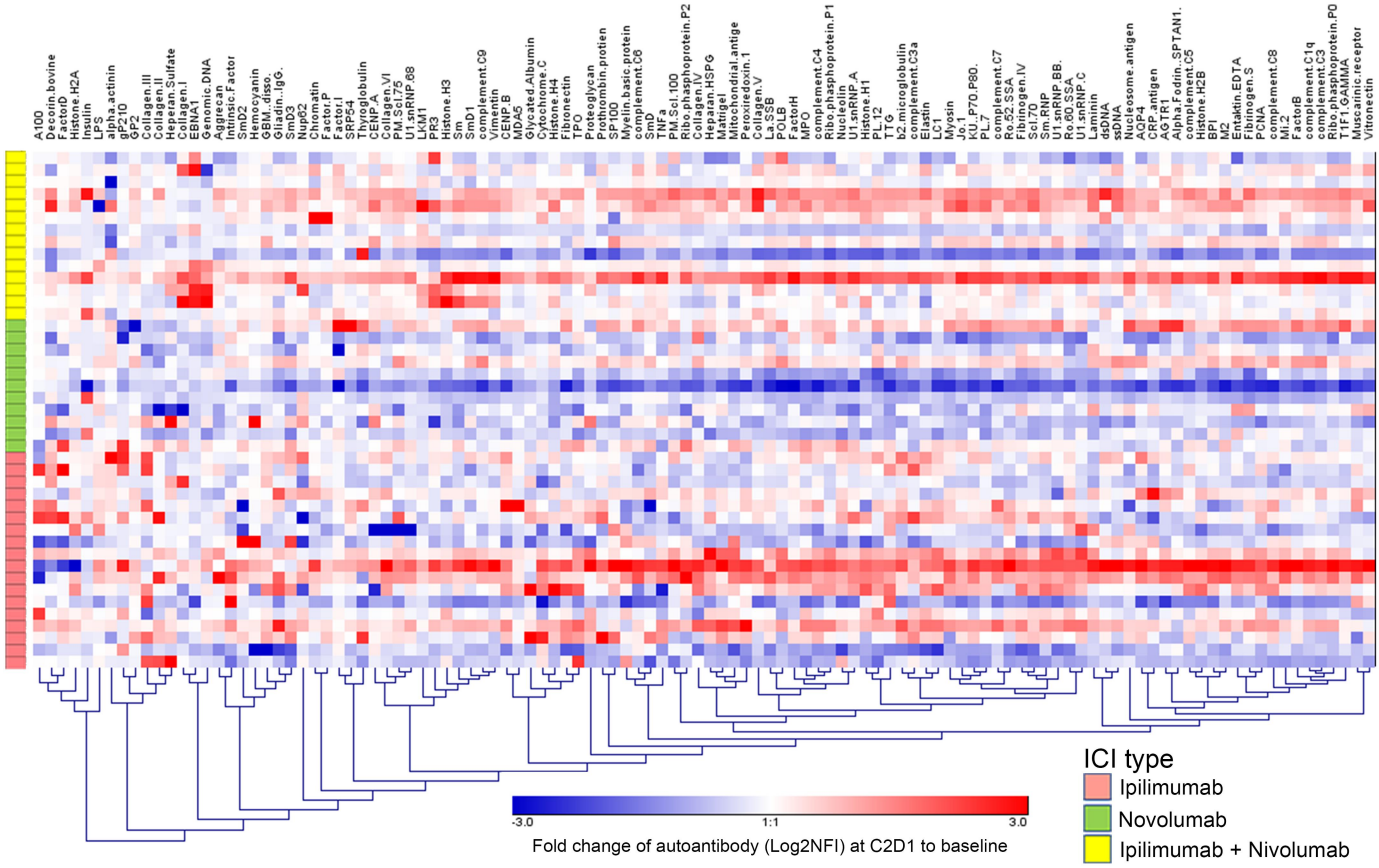

Figure 2. Post-treatment changes in 112 autoantibodies according to ICI type. Fold change of log2NFI(C2D1/BL) is shown. Within the heatmap, ipilimumab is shown in top rows, nivolumab in middle rows, and combination ipilimumab plus nivolumab in bottom rows.

that affect B-cell production of autoantibodies.[16] Conversely, PD-1 and PD-L1 are primarily involved in regulation of later stages of immune function, specifically T-cell effector function.[17] How do these mechanisms relate to our specific observations, namely dynamic autoantibody increases in the CTLA-4 and CTLA-4 + PD-1 groups, with largely unchanged or even decreased autoantibodies in the PD1 group? Preclinical studies have shown that selective deletion of CTLA-4 from B cells results in mice that spontaneously develop autoantibodies,[18] potentially explaining the autoantibody increases in our CTLA-4-treated patients. The effects of PD-1 inhibition on specific immune functions are largely reduced when combined with CTLA-4 inhibition,[19] which may support the similar autoantibody profiles we observed in the CTLA-4 and CTLA-4 + PD-1 groups. Because PD-1 inhibition primarily affects $CD8^+$ T cells, one might not necessarily expect a change in autoantibodies. As for the antibodies that decreased in the PD-1 group, almost all of them target collagen, and such autoantibodies have been noted to decrease rather than increase in inflammatory states.[20]

Different irAE types also appeared to have distinct autoantibody profiles, with liver irAE notable for decreased levels after ICI initiation. Clearly, the predominance of anti-PD1 cases (50%) within this group contributes to this association; however, the remaining cases from CTLA4-containing regimens tended to have either decreased, unchanged, or only modestly increased autoantibodies. To what extent these findings represent irAE mechanism cannot be determined from this study. Indeed, the relationship between specific autoantibody changes associated with ICI regimen and those associated with irAE was relatively modest. The small number of reports analyzing end-organ tissue from irAE generally describe cellular populations and do not evaluate antibody deposition.[21,22] We may gain some insight into the unexpected and counterintuitive decrease in autoantibodies we noted with certain irAE from studies in other autoimmune conditions. In rheumatoid arthritis, anti-collagen antibody titers decrease profoundly over time.[20] Similarly, in some patients with systemic lupus erythematosus, major declines in anti-dsDNA titers herald disease flares.[23] Whether these observations reflect antibody deposition into specific organs is not clear.

If irAE are eventually found to have either humoral immunity-dominant or -non-dominant pathophysiology, the clinical implications may be substantial, as immunosuppressants have varying effects on immune function.[24] Cyclosporin and tacrolimus target T cell proliferation. Rituximab specifically targets B cells. Mycophenolate has effects on both populations. Through effects on gene transcription and post-translational events, glucocorticoids broadly suppress inflammatory responses.[25]

Given the small sample size of this pilot study, we can infer little about specific autoantibodies. Among rash cases (the most common irAE), we noted increases in a number of

autoantibodies previously associated with autoimmune dermatologic conditions, including collagen, complement, and SSA.[26] Because our panel was designed to address a broad range of autoimmunity, it did not include certain autoantibodies strongly associated with dermatologic disease, such as those targeting epithelial basement membrane zone or cell surface.

We recognize the unique context of this study. Administration of multi-agent immunotherapy may introduce nuances in lymphoid malignancies. Because the underlying malignancy involves organ systems tightly linked with immune function, patients may exhibit immune dysregulation independent of therapy.[27] The treatments employed for these cancers frequently target lymphoid cells, thereby further affecting both humoral and cellular immune function. Brentuximab vedotin targets CD30, the physiologic expression of which is restricted to stimulated B immunoblasts in the germinal center and extrafollicular region. As precursors of plasma cells, immunoblasts might be expected to have a mechanistic link to antibody production. However, the three-week interval separating the two time-points in the present study may not be sufficient to demonstrate this effect.

The primary limitation of our study is the small size, which precludes detailed analysis of individual organ-specific irAE, evaluation of pre-treatment autoantibody levels as predictive biomarkers, or evaluation of survival and other efficacy outcomes. The three-week interval between baseline and post-treatment antibody determination may not capture later changes in these parameters, which have been previously described.[28] Nevertheless, because most irAE first occur after several weeks of ICI treatment, a pharmacodynamic biomarker with a relatively compressed timeline could prove useful for planning subsequent modifications to monitoring, supportive care, or treatment.

We also recognize that co-administration of brentuximab vedotin complicates interpretation of our findings, as some identified irAE may not represent immune-mediated toxicities. However, the most common toxicities of brentuximab – neuropathy and neutropenia – are largely attributable to its payload, the microtubule-disrupting agent monomethyl auristatin E, and are clinically distinct from common ICI-related toxicities. Rash occurs in about 10% of patients treated with brentuximab but is almost never high-grade, suggesting that most of our cases likely represent irAE. Hepatotoxicity is quite rare.[29] Similarly, the extent to which our findings in patients with lymphoma translate into the far larger solid tumor immuno-oncology population is not clear, as specific cancer types may have distinct risk of certain irAE (e.g., increased rates of pneumonitis in patients with lung cancer, increased rates of hepatitis in patients with liver cancer).[30,31]

A key strength of this study is the use of a robust autoantigen microarray. The array is not only enriched for autoantigens implicated in autoimmune diseases such as lupus and Sjogren's, but also features several antigens previously associated with irAE, including anti-TPO (thyroid), anti-glutamic acid decarboxylase (ICI-induced diabetes), and perinuclear antineutrophil cytoplasmic antibody (colitis). To optimize microarray reliability and data integrity, antigens were printed in duplicate but distributed randomly on microarray slides; data were batch-corrected and normalized using internal controls.

In conclusion, we found that the combinations of PD1- and CTLA4-targeting agents may have distinct effects on autoantibody and toxicity profiles. Pharmacodynamic testing of serial autoantibodies in larger cohorts treated with varying ICI regimens may improve our understanding of irAE risk and mechanism. Such information could eventually inform clinical management.

Acknowledgments

The E4412 study was coordinated by the Eastern Cooperative Oncology Group-American College of Radiology Imaging Network (ECOG-ACRIN) Cancer Research Group (Peter J. O'Dwyer, MD and Mitchell D. Schnall, MD, PhD, Group Co-Chairs) and supported by the National Cancer Institute of the National Institutes of Health under the following award numbers: U10CA180820, UG1CA233196, UG1CA233247, UG1CA233341, UG1CA233198, UG1CA233270, UG1CA233320, UG1CA233339, UG1CA233277, UG1CA232760. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The authors thank Ms. Dru Gray for assistance with manuscript preparation.

Disclosure statement

H.M-M.: intellectual property (U.S. patent applications 63/386,387, 63/382,972)
M.S.v.I.: none
F.F.: intellectual property (U.S. patent applications 17/045,482, 63/382,972)
J.L.: none
C.Z.: none
Y.X.: intellectual property (U.S. patent applications 17/045,482)
E.K.W.: intellectual property (U.S. patent 11,747,345; U.S. patent applications 17/045,482)
J.Y.P.: intellectual property (U.S. patent 11,747,345; U.S. patent applications 17/045,482, 63/382,972; co-founder and Chief Medical Officer, OncoSeer Diagnostics, LLC.)
B.S.K.: consulting/advisory boards (Abbvie, AstraZeneca, BeiGene, BMS, Genentech/Roche, Lilly, Seattle Genetics)
C.S.D.: grants (Seattle Genetics, BMS, Genentech), advisory boards/consulting (BMS, Seattle Genetics, Merck, Genentch)
D.E.G.: research funding (Astra-Zeneca, BerGenBio, Karyopharm, Novocure), stock ownership (Gilead, Medtronic, Walgreens), consulting/advisory Boards (Astra-Zeneca, Catalyst Pharmaceuticals, Daiichi-Sankyo, Elevation Oncology, Janssen Scientific Affairs, LLC, Jazz Pharmaceuticals, Regeneron Pharmaceuticals, Sanofi), intellectual property (U.S. patent 11,747,345; U.S. patent applications 17/045,482, 63/386,387, 63/382,972, 63/382,257; co-founder and Chief Medical Officer, OncoSeer Diagnostics, Inc.)

Funding

Funded in part by the National Cancer Institute [P30CA142543 supplement, UG1CA233302], the National Institute of Allergy and Infectious Disease [U01AI156189], an American Cancer Society-Melanoma Research Alliance Team Award [MRAT-18-114-01-LIB], a V Foundation Robin Roberts Cancer Survivorship Award [DT2019-007], the University of Texas Lung Cancer Specialized Program of Research Excellence [SPORE] [P50CA070907], a Physician-Scientist Institutional Award from the Burroughs Wellcome Fund, and the Harold C. Simmons Comprehensive Cancer Center Data Sciences Shared Resource [P30CA142543]. The funders were not involved in study design, conduct, or reporting.

ORCID

David E. Gerber http://orcid.org/0000-0002-7812-6741

Authors' contributions

H.M-M.: development of methodology; analysis and interpretation of data; writing (original draft); writing (review and editing); guarantor.
M.S.v.I.: analysis and interpretation of data; writing (original draft); writing (review and editing).
F.F.: development of methodology; analysis and interpretation of data; writing (original draft); writing (review and editing); study supervision.
J.L.: analysis and interpretation of data; writing (review and editing).
C.Z.: analysis and interpretation of data; writing (review and editing).
Y.X.: development of methodology; analysis and interpretation of data; writing (review and editing); study supervision.
E.K.W.: development of methodology; analysis and interpretation of data; writing (review and editing).
B.S.K.: design of, accrual of patients to, and collection of data for the E4412 trial; writing (review and editing).
C.S.D.: conception and design of, accrual of patients to, and collection of data for the E4412 trial; analysis and interpretation of data; writing (review and editing).
D.E.G.: conception and design; analysis and interpretation of data; writing (original draft); writing (review and editing); funding acquisition; study supervision; guarantor.

Data availability statement

Data from Eastern Cooperative Oncology Group-American College of Radiology Imaging Network (ECOG-ACRIN) clinical trials are available to researchers. Deidentified data, including data dictionaries, are available for request per the ECOG-ACRIN Data Sharing Policy after publication of the primary publication. Access to supplemental materials will be considered at time of request. If the request is approved and if there are no restrictions to ECOG-ACRIN sharing data, a data use agreement between the requestor's institution and ECOG-ACRIN must be in place before requested data can be released.

References

1. Ribas A, Hodi FS, Callahan M, Konto C, Wolchok J. Hepatotoxicity with combination of vemurafenib and ipilimumab. N Engl J Med. 2013;368(14):1365–1366. doi:10.1056/NEJMc1302338.
2. Ahn MJ, Cho BC, Ou X, Walding A, Dymond AW, Ren S, Cantarini M, Jänne PA. Osimertinib plus durvalumab in patients with EGFR-Mutated, advanced NSCLC: a phase 1b, open-label, multicenter trial. J Thorac Oncol. 2022;17(5):718–723. doi:10.1016/j.jtho.2022.01.012.
3. Garassino MC, Gadgeel S, Speranza G, Felip E, Esteban E, Dómine M, Hochmair MJ, Powell SF, Bischoff HG, Peled N. et al. Pembrolizumab plus pemetrexed and platinum in nonsquamous non–small-Cell lung cancer: 5-year outcomes from the phase 3 KEYNOTE-189 study. J Clin Oncol. 2023;41(11):1992–1998. doi:10.1200/JCO.22.01989.
4. Arbuckle MR, McClain MT, Rubertone MV, Scofield RH, Dennis GJ, James JA, Harley JB. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N Engl J Med. 2003;349(16):1526–1533. doi:10.1056/NEJMoa021933.
5. Ghosh N, Chan KK, Jivanelli B, Bass AR. Autoantibodies in patients with immune-related adverse events from checkpoint inhibitors: a systematic literature review. J Clin Rheumatol. 2022;28(2):e498–e505. doi:10.1097/RHU.0000000000001777.
6. de Moel EC, Rozeman EA, Kapiteijn EH, de Moel EC, Verdegaal EME, Grummels A, Bakker JA, Huizinga TWJ, Haanen JB, Toes REM, van der Woude D. Autoantibody development under treatment with immune-checkpoint inhibitors. Cancer Immunol Res. 2019;7(1):6–11. doi:10.1158/2326-6066.CIR-18-0245.
7. Gowen MF, Giles KM, Simpson D, Tchack J, Zhou H, Moran U, Dawood Z, Pavlick AC, Hu S, Wilson MA. et al. Baseline antibody profiles predict toxicity in melanoma patients treated with immune checkpoint inhibitors. J Transl Med. 2018;16(1):82. doi:10.1186/s12967-018-1452-4.
8. Toi Y, Sugawara S, Sugisaka J, Ono H, Kawashima Y, Aiba T, Kawana S, Saito R, Aso M, Tsurumi K. et al. Profiling preexisting antibodies in patients treated with anti–PD-1 therapy for advanced non–small cell lung cancer. JAMA Oncol. 2019;5(3):376–383. doi:10.1001/jamaoncol.2018.5860.
9. Diefenbach CS, Hong F, Ambinder RF, Cohen JB, Robertson MJ, David KA, Advani RH, Fenske TS, Barta SK, Palmisiano ND. et al. Ipilimumab, nivolumab, and brentuximab vedotin combination therapies in patients with relapsed or refractory Hodgkin lymphoma: phase 1 results of an open-label, multicentre, phase 1/2 trial. Lancet Haematol. 2020;7(9):e660–e670. doi:10.1016/S2352-3026(20)30221-0.
10. Ghosh N, Postow M, Zhu C, Jannat-Khah D, Li Q-Z, Vitone G, Chan KK, Bass AR. Lower baseline autoantibody levels are associated with immune-related adverse events from immune checkpoint inhibition. J Immunother Cancer. 2022;10(1):e004008. doi:10.1136/jitc-2021-004008.
11. Li QZ, Zhou J, Wandstrat AE, Carr-Johnson F, Branch V, Karp DR, Mohan C, Wakeland EK, Olsen NJ. Protein array autoantibody profiles for insights into systemic lupus erythematosus and incomplete lupus syndromes. Clin Exp Immunol. 2007;147(1):60–70. doi:10.1111/j.1365-2249.2006.03251.x.
12. Lee EC, Whitehead AL, Jacques RM, Julious SA. The statistical interpretation of pilot trials: should significance thresholds be reconsidered? BMC Med Res Methodol. 2014;14(1):41. doi:10.1186/1471-2288-14-41.
13. Tahir SA, Gao J, Miura Y, Blando J, Tidwell RSS, Zhao H, Subudhi SK, Tawbi H, Keung E, Wargo J. et al. Autoimmune antibodies correlate with immune checkpoint therapy-induced toxicities. Proc Natl Acad Sci USA. 2019;116(44):22246–22251. doi:10.1073/pnas.1908079116.
14. Barth DA, Stanzer S, Spiegelberg J, Bauernhofer T, Absenger G, Posch F, Lipp R, Halm M, Szkandera J, Balic M. et al. Evaluation of autoantibodies as predictors of treatment response and immune-related adverse events during the treatment with immune checkpoint inhibitors: a prospective longitudinal pan-cancer study. Cancer Med. 2022;11(16):3074–3083. doi:10.1002/cam4.4675.
15. Johannet P, Liu W, Fenyo D Wind-Rotolo, M., Krogsgaard, M., Mehnert, J.M., Weber, J.S., Zhong, J. and Osman, I. Baseline serum autoantibody signatures predict recurrence and toxicity in melanoma patients receiving adjuvant immune checkpoint blockade. Clin Cancer Res. 2022;28(18):4121–4130. doi:10.1158/1078-0432.CCR-22-0404.
16. Hossen MM, Ma Y, Yin Z, Xia Y, Du J, Huang JY, Huang JJ, Zou L, Ye Z, Huang Z. et al. Current understanding of CTLA-4: from mechanism to autoimmune diseases. Front Immunol. 2023;14:1198365. doi:10.3389/fimmu.2023.1198365.
17. Francisco LM, Sage PT, Sharpe AH. The PD-1 pathway in tolerance and autoimmunity. Immunol Rev. 2010;236:219–242. doi:10.1111/j.1600-065X.2010.00923.x.
18. Yang Y, Li X, Ma Z, Wang C, Yang Q, Byrne-Steele M, Hong R, Min Q, Zhou G, Cheng Y. et al. CTLA-4 expression by B-1a B cells is essential for immune tolerance. Nat Commun. 2021;12(1):525. doi:10.1038/s41467-020-20874-x.
19. Wei SC, Sharma R, Anang NAS, Levine JH, Zhao Y, Mancuso JJ, Setty M, Sharma P, Wang J, Pe'er D. et al. Negative Co-stimulation constrains T cell differentiation by imposing boundaries on possible cell states. Immunity. 2019;50(4):1084–1098 e10. doi:10.1016/j.immuni.2019.03.004.
20. Pereira RS, Black CM, Duance VC, Jones VE, Jacoby RK, Welsh KI. Disappearing collagen antibodies in rheumatoid

arthritis. Lancet. 1985;326(8453):501–502. doi:10.1016/s0140-6736(85)90436-2.
21. Imoto K, Kohjima M, Hioki T, Kurashige T, Kurokawa M, Tashiro S, Suzuki H, Kuwano A, Tanaka M, Okada S. et al. Clinical features of liver injury induced by immune checkpoint inhibitors in Japanese patients. Can J Gastroenterol Hepatol. 2019;2019:1–12. doi:10.1155/2019/6391712.
22. Reschke R, Shapiro JW, Yu J, Rouhani SJ, Olson DJ, Zha Y, Gajewski TF. Checkpoint blockade–induced dermatitis and colitis are dominated by tissue-resident memory T cells and Th1/Tc1 cytokines. Cancer Immunol Res. 2022;10(10):1167–1174. doi:10.1158/2326-6066.CIR-22-0362.
23. Yeo AL, Kandane-Rathnayake R, Koelmeyer R, Golder V, Louthrenoo W, Chen Y-H, Cho J, Lateef A, Hamijoyo L, Luo S-F. et al. SMART-SLE: serology monitoring and repeat testing in systemic lupus erythematosus—an analysis of anti-double-stranded DNA monitoring. Rheumatology (Oxford). 2024;63(2):525–533. doi:10.1093/rheumatology/kead231.
24. Meneghini M, Bestard O, Grinyo JM. Immunosuppressive drugs modes of action. Best Pract Res Clin Gastroenterol. 2021;54-55:101757. doi:10.1016/j.bpg.2021.101757.
25. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids–new mechanisms for old drugs. N Engl J Med. 2005;353(16):1711–1723. doi:10.1056/NEJMra050541.
26. Leiferman KM, Snook JP, Khalighi MA, Kuechle MK, Zone JJ. Diagnostics for dermatologic diseases with autoantibodies. J Appl Lab Med. 2022;7(1):165–196. doi:10.1093/jalm/jfab147.
27. Pelosof LC, Gerber DE. Paraneoplastic syndromes: an approach to diagnosis and treatment. Mayo Clin Proc. 2010;85(9):838–854. doi:10.4065/mcp.2010.0099.
28. Khan S, von Itzstein MS, Lu R, Bermas BL, Karp DR, Khan SA, Fattah FJ, Park JY, Saltarski JM, Gloria-McCutchen Y. et al. Late-onset immunotherapy toxicity and delayed autoantibody changes: checkpoint inhibitor–induced Raynaud's-like phenomenon. Oncologist. 2020;25(5):e753–e757. doi:10.1634/theoncologist.2019-0666.
29. Forero-Torres A, Bartlett NL, Berryman RB, Chen R, Matous JV, Fanale MA, O'Connor OA, Olshefski R, Smith SE, Huebner D. et al. Extended treatment with brentuximab vedotin in patients with relapsed or refractory CD30-positive hematological malignancies. Leuk Lymphoma. 2015;56(4):1151–1153. doi:10.3109/10428194.2014.951843.
30. Wu J, Hong D, Zhang X, Lu X, Miao J. PD-1 inhibitors increase the incidence and risk of pneumonitis in cancer patients in a dose-independent manner: a meta-analysis. Sci Rep. 2017;7(1):44173. doi:10.1038/srep44173.
31. Fu J, Li WZ, McGrath NA, Lai CW, Brar G, Xiang Y-Q, Xie C. Immune checkpoint inhibitor associated hepatotoxicity in primary liver cancer versus other cancers: a systematic review and meta-analysis. Front Oncol. 2021;11:650292. doi:10.3389/fonc.2021.650292.