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## ORIGINAL RESEARCH



# CTLA-4 Pathway Is Instrumental in Giant Cell Arteritis

Paul Régnier<sup>1</sup>, Alexandre Le Joncour<sup>2\*</sup>, Anna Maciejewski-Duval<sup>3\*</sup>, Guillaume Darrasse-Jèze<sup>4</sup>, Charles Dolladille<sup>5</sup>, Wouter C. Meijers, Lisa Bastarache, Pierre Fouret, Patrick Bruneval, Floriane Arbaretaz<sup>6</sup>, Célia Sayetta, Ana Márquez, Michelle Rosenzweig<sup>7</sup>, David Klatzmann<sup>8</sup>, Patrice Cacoub<sup>9</sup>, Javid J. Moslehi<sup>10</sup>, Joe-Elie Salem<sup>11\*</sup>, David Saadoun<sup>12\*</sup>

**BACKGROUND:** Giant cell arteritis (GCA) causes severe inflammation of the aorta and its branches and is characterized by intense effector T-cell infiltration. The roles that immune checkpoints play in the pathogenesis of GCA are still unclear. Our aim was to study the immune checkpoint interplay in GCA.

**METHODS:** First, we used VigiBase, the World Health Organization international pharmacovigilance database, to evaluate the relationship between GCA occurrence and immune checkpoint inhibitors treatments. We then further dissected the role of immune checkpoint inhibitors in the pathogenesis of GCA, using immunohistochemistry, immunofluorescence, transcriptomics, and flow cytometry on peripheral blood mononuclear cells and aortic tissues of GCA patients and appropriated controls.

**RESULTS:** Using VigiBase, we identified GCA as a significant immune-related adverse event associated with anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) but not anti-PD-1 (anti-programmed death-1) nor anti-PD-L1 (anti-programmed death-ligand 1) treatment. We further dissected a critical role for the CTLA-4 pathway in GCA by identification of the dysregulation of CTLA-4-derived gene pathways and proteins in CD4<sup>+</sup> (cluster of differentiation 4) T cells (and specifically regulatory T cells) present in blood and aorta of GCA patients versus controls. While regulatory T cells were less abundant and activated/suppressive in blood and aorta of GCA versus controls, they still specifically upregulated CTLA-4. Activated and proliferating CTLA-4<sup>+</sup> Ki-67<sup>+</sup> regulatory T cells from GCA were more sensitive to anti-CTLA-4 (ipilimumab)-mediated in vitro depletion versus controls.

**CONCLUSIONS:** We highlighted the instrumental role of CTLA-4 immune checkpoint in GCA, which provides a strong rationale for targeting this pathway.

**GRAPHIC ABSTRACT:** A [graphic abstract](#) is available for this article.

**Key Words:** giant cell arteritis ■ humans ■ immune checkpoint inhibitors ■ T-lymphocytes, regulatory ■ vasculitis

## In This Issue, see p 295 | Meet the First Author, see p 296

**G**iant cell arteritis (GCA) is the most common systemic vasculitis involving the aorta and is associated with an increased risk of thoracic and abdominal aneurysms. Vascular complications such as stroke and aortic aneurysm or dissection account for the main causes of death related to the disease.<sup>1</sup> Pathologically, GCA is characterized by CD4<sup>+</sup> (cluster of differentiation 4) T cells and macrophages, which can undergo granulomatous organization with the formation of giant cells.

Immune infiltration can penetrate from the adventitia tunica into the media, leading to the destruction of vessel wall integrity and ease of formation of aneurysms.<sup>2</sup> Infiltrating effector T cells exhibit Th1 (T helper 1)/Th17 (T helper 17) unbalanced immune polarization with a decreased proportion of regulatory T cells (Tregs).<sup>3,4</sup> In addition, oligoclonal T cells have been isolated from spatially distinct GCA lesions,<sup>5</sup> which is highly suggestive for antigen-driven T-cell activation.

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## Novelty and Significance

### What Is Known?

- Giant cell arteritis (GCA) is characterized by intense infiltration of the vessel by Th1 (T helper 1)/Th17 (T helper 17) effector cells with a decreased proportion of regulatory T cells (Tregs).
- Emerging data also suggest an important contribution from immune-cell inhibitory signaling in GCA.

### What New Information Does This Article Contribute?

- Using VigiBase, the World Health Organization international pharmacovigilance database, we identified GCA as a specific drug toxicity associated with CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) inhibitors used for cancer therapy.
- We then identified a strong upregulation of CTLA-4–derived gene pathways and proteins in CD4<sup>+</sup> T cells (and specifically Tregs) in blood and aorta tissue of patients with GCA.
- Although Tregs from GCA show a less suppressive phenotype, the remaining activated CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs are highly sensitive to anti-CTLA-4–mediated in vitro depletion.

In older adults, GCA is the most frequent large vessel vasculitis. Arterial consequences include stenoses, obstruction, and aneurysm that can lead to arterial insufficiency, need for procedures, and blindness. The immune T-cell infiltration of the vessel wall in GCA includes heightened Th1/Th17 effector cells with decreased proportion of Tregs. Though immune-cell activation clearly contributes to arterial pathology in GCA, emerging data suggest an important contribution of altered immune-cell inhibitory signaling. In our work, we identify a novel association of the increasingly used cancer agents, CTLA-4 inhibitors, with the occurrence of GCA. To explore the potential mechanisms linking CTLA-4 inhibitors with vasculitis, we demonstrated upregulation of CTLA-4 in CD4<sup>+</sup> T cells, particularly Tregs, in circulation and aortic tissue of patients with GCA compared with controls. Although Tregs from GCA showed a weaker suppressor phenotype compared with controls, the remaining activated CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs from GCA were highly sensitive to anti-CTLA-4–mediated in vitro depletion. This study paves the way for modulating the CTLA-4 signaling pathway in GCA.

### Nonstandard Abbreviations and Acronyms

<b>aGCA</b>	active giant cell arteritis
<b>CTLA-4</b>	cytotoxic T-lymphocyte-associated protein-4
<b>GCA</b>	giant cell arteritis
<b>HD</b>	healthy donor
<b>ICI</b>	immune checkpoint inhibitor
<b>IL</b>	interleukin
<b>iGCA</b>	inactive giant cell arteritis
<b>irAE</b>	immune-related adverse event
<b>PBMC</b>	peripheral blood mononuclear cell
<b>TAK</b>	Takayasu arteritis
<b>Tconv</b>	conventional T cell
<b>Treg</b>	regulatory T cell

While dysregulated immune-cell activation undoubtedly contributes to disease pathogenesis in GCA, emerging data also suggest an important contribution from immune-cell inhibitory signaling. The immune system requires regulation to ensure an equilibrium between tolerance against self-antigens and protection against tumorigenesis.<sup>6,7</sup> Immune checkpoint-mediated pathways are critical in these processes<sup>8,9</sup> and include

CTLA-4 (cytotoxic T-lymphocyte-associated protein-4), expressed on T cells and other immune effector cells, which binds CD80 and CD86, expressed by antigen-presenting cells.<sup>8</sup> CTLA-4 is an inhibitory molecule that is predominantly expressed by T cells and especially by Tregs and memory CD4<sup>+</sup> conventional T cells (Tconv) as compared with naive CD4<sup>+</sup> Tconv.<sup>10,11</sup> It competes with CD28, the primary source of positive costimulation of T-cell activation in the lymphoid organs. Furthermore, in addition to its competition activity, CTLA-4 binding to CD28 is also sought to directly induce inhibitory signals able to counteract the regular CD28-CD80/CD86 ligation, as well as to deeply disrupt the associated CD28 downstream signaling pathway.<sup>11,12</sup> For these reasons, CTLA-4 is both considered as a robust marker of CD4<sup>+</sup> Tconv sustained activation/anergy and Treg functionality. In the periphery, important immune checkpoint proteins include PD-1 and its ligands (PD-L1 and PD-L2).<sup>9,12,13</sup> Overall, CTLA-4/CD80-CD86 and PD-1/PD-L1 axes are important suppressors of immune responses. Human genetic variants of *CTLA4* are associated with autoimmune disorders: heterozygous *CTLA4* mutations in patients are associated with Tregs dysregulation, effector T cells hyperactivation, and lymphocytic tissue infiltration.<sup>14,15</sup> This inhibitory role for CTLA-4 and PD-1/PD-L1 led to the emergence of immune checkpoint inhibitors (ICIs), notably incarnated by monoclonal

antibodies against CTLA-4, PD-1, or PD-L1, which have revolutionized cancer treatment. However, ICI treatments can also result in immune-related adverse events (irAEs) and autoimmune-like toxicities that can affect any organ. ICI-associated irAEs include a quickly growing number of systemic vasculitis,<sup>16,17</sup> as well as histologically confirmed GCA cases.<sup>18,19</sup>

The roles that immune checkpoints play in pathogenesis of GCA and other vasculitis is only beginning to be realized. Zhang et al<sup>20</sup> showed that in GCA, PD-1/PD-L1 axis plays a tissue protective role in the vasculature with impaired PD-L1 signaling possibly contributing to the disease. While PD-(L)1 is generally considered as a peripheral immune checkpoint, CTLA-4 is thought to primarily inhibit T-cell and immune-cell signaling in the lymph nodes.<sup>9,12</sup> Here, we found that CTLA-4 signaling plays a critical role in GCA. Stemming from a clinical and epidemiological observation that GCA is a significant irAE from anti-CTLA-4 treatment but not anti-PD-(L)1 treatment, we further dissected a critical role for CTLA-4 in the pathogenesis of GCA using immunohistochemistry, immunofluorescence, transcriptomics, and flow cytometry on peripheral blood mononuclear cells (PBMCs) and aortic tissues of GCA patients and appropriated controls.

## METHODS

### Data Availability

Transcriptome data are publicly available and hosted on Gene Expression Omnibus (GEO) repository under the identifier GSE236367. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

All other data presented in this study (VigiBase, flow cytometry, immunohistochemistry, and immunofluorescence) are available upon reasonable request to D.S. Reuse is permitted if data are not altered in any way and if original authors are correctly cited in the subsequent publications.

### Code Availability

Analyses involving the R software were performed with only open-source and publicly available packages, either hosted on the Comprehensive R Archive Network (for uwot package) or Bioconductor (for flowStats, ggcyto, flowCore, GSVA, illuminaio, limma, and sva packages) repositories. The code used was directly adapted from the associated documentations, which can be found on [https://cran.r-project.org/web/packages/available\\_packages\\_by\\_name.html](https://cran.r-project.org/web/packages/available_packages_by_name.html) or <https://bioconductor.org/packages/devel/bioc/>. Analyses involving the Fiji software were performed with macro script calling only open-source code from built-in plug-ins, which was directly adapted from the official Fiji and ImageJ documentations ([https://imagej.net/Introduction\\_into\\_Macro\\_Programming](https://imagej.net/Introduction_into_Macro_Programming)).

### Experimental and Study Design

The study population consisted of 44 GCA patients (66.67% women; mean age  $\pm$ SD, 72.77 $\pm$ 10.67 years; and median age,

72.5 years [interquartile range, 64–81]) who fulfilled the international criteria for GCA.<sup>21</sup> Among them, 34 presented an active disease (active GCA [aGCA]) and 10 an inactive disease (inactive GCA [iGCA]). These patients were either newly diagnosed or already followed in our internal medicine department. More information about clinical and demographic features of GCA patients is available in Table 1. Samples from 45 sex- and age-matched healthy donors (HD; 62.22% women; mean age, 65.67 $\pm$ 20.75 years; and median age, 68 years [interquartile range, 50.5–84]) were obtained from the Établissement Français du Sang facility. Fresh blood samples were immediately put on Ficoll layer and centrifuged for 20 minutes at 2200 rpm, before PBMCs were extracted and frozen in liquid nitrogen in an 80%/20% fetal calf serum/dimethyl sulfoxide mix. All the experiments of this study were performed on independent samples, originating from the different groups of interest. No repeated measurements were performed on the same samples. The study itself was approved by our institutional ethics review board, and the use of confidential electronically processed patient data was approved by the French National

**Table 1. Demographic and Clinical Features of the Studied GCA Cohort**

Parameters	GCA (n=44)
Demographic features	
Age, y; mean $\pm$ SD	72.77 $\pm$ 10.67 (n=44)
Age, y; median (IQR)	72.5 (64–81; n=44)
Female gender, n/n <sub>total</sub> (%)	29/44 (65.9%)
Geographic origin, n/n <sub>total</sub> (%)	
Europe	40/44 (90.9%)
North Africa	4/44 (9.1%)
Clinical features	
Active disease, n/n <sub>total</sub> (%)	34/44 (77.3%)
General symptoms, n/n <sub>total</sub> (%)	
Fever*	9/23 (39.1%)
Jaw claudication*	13/27 (48.2%)
Headache*	21/28 (75%)
Scalp sensitivity*	10/23 (43.5%)
Cough*	2/23 (8.7%)
Stroke,* n/n <sub>total</sub> (%)	4/29 (13.8%)
Aortitis,* n/n <sub>total</sub> (%)	17/32 (53.1%)
Stenosis*	3/12 (25%)
Aneurysms*	5/17 (29.4%)
Dissection*	0/12 (0%)
Surgical repair*	2/12 (16.7%)
Optic neuropathy,* n/n <sub>total</sub> (%)	6/34 (17.6%)
Polymyalgia rheumatica,* n/n <sub>total</sub> (%)	15/29 (51.7%)
C-reactive protein level,* mg/L; mean $\pm$ SD	36.7 $\pm$ 35.2 (n=35)
ESR,* mm/h; mean $\pm$ SD	33.4 $\pm$ 35.1 (n=10)

Several demographic and clinical features of the studied GCA cohort were collected and presented in the table. Data are displayed as the number of cases among the total number of cases for which information was available. When specified, some data are shown as mean $\pm$ SD or as median with IQR. ESR indicates Erythrocyte Sedimentation Rate; GCA, giant cell arteritis; and IQR, interquartile range.

\*The presence of missing values.

**Table 2. RORs and Their 95% CIs, Comparing Selected Vasculitis and Different ICI Treatment Regimens**

Total number of reports	Overall immune checkpoint inhibitors* (ICI, n=84 759)			Full database (starting 2008, n=18033 220)	ROR (95% CI), Mono- $\alpha$ CTLA-4 vs Mono- $\alpha$ PD-(L)1	ROR (95% CI), Comb vs Mono	ROR (95% CI), ICI vs full base
	Mono† (n=75 804)		Combi (n=8955)				
	Mono- $\alpha$ PD-(L)1‡ (n=65 375)	Mono- $\alpha$ CTLA-4§ (n=10 429)					
No. of vasculitis reports by subgroup							
GCA	18 (0.03%)	10 (0.1%)	3 (0.03%)	767 (<0.01%)	3.48 (1.61–7.55)¶	0.91 (0.28–2.98)	8.56 (5.98–12.26)¶
Renal vasculitis	3 (<0.01%)	1 (0.01%)	0 (0%)	161 (<0.01%)	2.09 (0.22–20.09)	...	5.26 (1.95–14.19)¶
Retinal vasculitis	7 (0.01%)	1 (0.01%)	0 (0%)	316 (<0.01%)	0.90 (0.11–7.28)	...	5.36 (2.66–10.81)¶
ANCA vasculitis	15 (0.02%)	2 (0.02%)	5 (0.06%)	2,265 (0.01%)	0.84 (0.19–3.66)	2.49 (0.92–6.75)	2.06 (1.35–3.13)¶
Aortitis	8 (0.01%)	1 (0.01%)	0 (0%)	181 (<0.01%)	0.78 (0.10–6.27)	...	10.53 (5.39–20.57)¶
CNS vasculitis	10 (0.02%)	1 (0.01%)	0 (0%)	386 (<0.01%)	0.63 (0.08–4.90)	...	6.04 (3.31–10.99)¶

Different comparisons were performed from VigiBase and are shown in distinct columns: immune checkpoint inhibitors (anti-CTLA-4 or anti-PD-1 [anti-programmed death-1] or anti-PD-L1 [anti-programmed death-ligand 1]) vs full database (seventh column), combined immunotherapy vs mono-immunotherapy (sixth column) and anti-PD-1 or anti-PD-L1 mono-immunotherapies vs anti-CTLA-4 mono-immunotherapy (fifth column). The time period of analysis was from January 1, 2008, to June 1, 2020. Of note, first reports associated with ICI started in 2008. ANCA indicates antineutrophil cytoplasmic antibody; CNS, central nervous system; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; GCA, giant cell arteritis; ICI, immune checkpoint inhibitor; ICSR, individual case safety report; PD-1, programmed death-1; and ROR, reporting odds ratio.

\*Any ICSR related to atezolizumab, avelumab, cemiplimab, durvalumab, ipilimumab, nivolumab, pembrolizumab, or tremelimumab when used either alone or in combination (denoted as overall immune checkpoint inhibitors).

†Any report related to any of the 8 previously cited drugs only when used in monotherapy (denoted as Mono).

‡Any report related to atezolizumab, avelumab, cemiplimab, durvalumab, nivolumab, or pembrolizumab only when used in monotherapy (denoted as Mono-anti-PD-[L]1).

§Any report related to ipilimumab or tremelimumab only when used in monotherapy (denoted as Mono-anti-CTLA-4).

¶Any report related to at least 1 drug from anti-PD-(L)1 inhibitors combined with an anti-CTLA-4 (denoted as Comb).

¶¶Significant overreporting ( $P < 0.05$ ).

Commission for Data Protection and Liberties (Commission Nationale de l'Informatique et des Libertés, authorization protocol number 1867484). All patients gave informed and written consent. For further details about the main reagents we used throughout this article, please see the Major Resources Table in the [Supplemental Material](#).

### Immunolabeling of Aortic Tissue Sections

Immunohistochemistry and immunofluorescence protocols were performed on fixed, 5- $\mu$ m paraffin-embedded aorta sections obtained from biopsies of GCA and noninflammatory control aneurysms (CTRL). Full details about these labeling and acquisition protocols and data processing and analysis are available in the [Supplemental Material and Methods](#).

### Flow Cytometry Data Generation

PBMCs from either HD or GCA were thawed and stained with 3 distinct panels of antibodies following a standard flow cytometry-compatible staining protocol, then immediately acquired using CytoFLEX (Beckman Coulter). The associated gating strategies are shown in [Figures S4 through S6](#). Further details about the panels and protocols are available in the [Supplemental Material and Methods](#).

### Statistical Analysis

For [Figures 2B, 3A through 3E, 4D, 5A, 5C through 5G](#) and [Figures S1 through S3](#), differences between clinical groups of interest were assessed using the nonparametric 2-tailed

Mann-Whitney unpaired tests. For [Figures 3A, 3C through 3E, 5C through 5F](#) and [Figures S2A, S2B, S2G, S3B, and S3C](#), differences between cell populations of interest were assessed using nonparametric 2-tailed Wilcoxon matched-pairs signed-rank tests. For [Figure 4A through 4C](#), moderated 2-tailed  $t$  tests from limma Bioconductor R package with Benjamini-Hochberg  $P$  value correction for multiple tests were used. ns,  $P \geq 5 \times 10^{-2}$ ; \* $P < 5 \times 10^{-2}$ ; \*\* $P < 1 \times 10^{-2}$ ; \*\*\* $P < 1 \times 10^{-3}$ ; \*\*\*\* $P < 1 \times 10^{-4}$ . When no information about significance is displayed on the figure, it means that no statistical tests have been performed at all. When violin plots are used, horizontal plain bars represent median and horizontal dashed bars represent Q1 and Q3 quartiles. Of note, no  $P$  value adjustment for multiple testing was performed across tests of the different experiments/analyses presented. The full details about the performed tests, the compared groups, their associated mean/median and SEM/interquartile range, as well as the precise  $P$  values and derived summaries (and more), are available in [Table S1](#).

## RESULTS

### GCA Cases Are a Significant irAE From Anti-CTLA-4 but Not Anti-PD-(L)1 Treatment in VigiBase

To determine whether there is a statistical association between ICI treatments and GCA, we used the international pharmacovigilance database VigiBase to analyze the association between different ICI regimens and

**Table 3. Medical Dictionary for Drug Regulatory Activities Terms Used to Classify the Different Types of Vasculitis Used in VigiBase**

Vasculitis grouping	Preferred MedDRA Term (PT) levels
Large vessel vasculitis	
GCA	Giant cell arteritis (PT)
TAK	Takayasu's arteritis (PT)
Aortitis	Aortitis (PT)
Medium vessel vasculitis	
Polyarteritis nodosa	Vasculitis gastrointestinal (PT) or polyarteritis nodosa (PT)
Kawasaki disease	Kawasaki's disease (PT)
Small vessel vasculitis	
Antineutrophil cytoplasmic antibody-associated vasculitis	Anti-neutrophil cytoplasmic antibody positive vasculitis (PT) or eosinophilic granulomatosis with polyangiitis (PT) or granulomatosis with polyangiitis (PT) or antineutrophil cytoplasmic antibody increased (PT) or antineutrophil cytoplasmic antibody positive (PT) or microscopic polyangiitis (PT)
Henoch-Schonlein purpura (IgA vasculitis)	Henoch-Schonlein purpura (PT) or Henoch-Schonlein purpura nephritis (PT)
Cryoglobulinaemia	Cryoglobulinaemia (PT) or cryoglobulins present (PT)
Antiglomerular basement membrane (anti-GBM) disease	Anti-glomerular basement membrane disease (PT) or Goodpasture's syndrome (PT)
Skin vasculitis	Cutaneous vasculitis (PT) or hypersensitivity vasculitis (PT) or urticarial vasculitis (PT) or vasculitic rash (PT) or chronic pigmented purpura (PT) or erythema induratum (PT) or nodular vasculitis (PT) or vascular purpura (PT) or palpable purpura (PT) or type 2 lepra reaction (PT) or segmented hyalinising vasculitis (PT)
Multiple vessel type vasculitis	
Behcet-associated syndromes	Behcet's syndrome (PT) or MAGIC (mouth and genital ulcers with inflamed cartilage) syndrome (PT)
Cogan syndrome	Cogan's syndrome (PT)
Vasculitis associated with systemic diseases	
Rheumatological vasculitis	Lupus vasculitis (PT) or rheumatoid vasculitis (PT)
Viral vasculitis	Viral vasculitis (PT)
Miscellaneous vasculitis	
Thromboangiitis obliterans	Thromboangiitis obliterans (PT)
Coronaritis	Arteritis coronary (PT)
Renal vasculitis	Renal arteritis (PT) or renal vasculitis (PT)
Central nervous system vasculitis	Central nervous system vasculitis (PT) or cerebral arteritis (PT)
Retinal vasculitis	Ocular vasculitis (PT) or retinal vasculitis (PT)
Pulmonary vasculitis	Pulmonary vasculitis (PT)
Administration-site vasculitis	Administration site vasculitis (PT) or application site vasculitis (PT) or infusion site vasculitis (PT) or injection site vasculitis (PT)

This table shows the actual MedDRA (version 23.1) terms used for the classification of the analyzed vasculitis on VigiBase. GCA indicates giant cell arteritis; and MedDRA, Medical Dictionary for Drug Regulatory Activities.

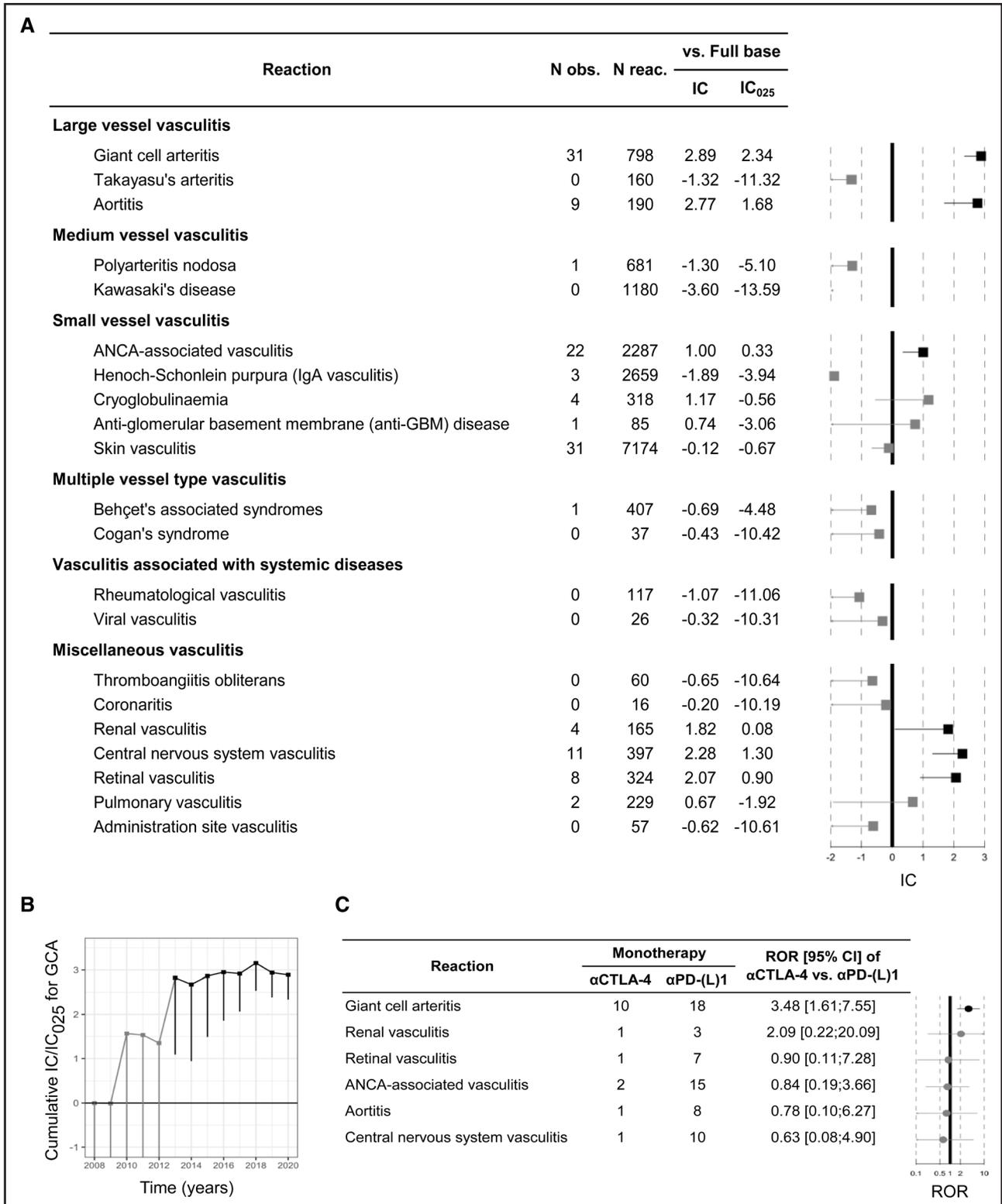
different types of vasculitis reported as an irAE. Details of these analyses are summarized in Table 2. Of note, we used different queries for the different types of vasculitis according to the Medical Dictionary for Drug Regulatory Activities (MedDRA) which are fully detailed in Table 3. ICI regimens assessed were anti-CTLA-4 or anti-PD-1 (anti-programmed death-1) or anti-PD-L1 (anti-programmed death-ligand 1) (as monotherapies or combinations). We observed that only 6 of 21 types of vasculitis were significantly overreported following ICI treatment versus the full database. Among them, GCA had both the highest absolute number of cases and magnitude of disproportional associations (Figure 1A; Table 2), which reached significance in 2013 (Figure 1B). Of note, Takayasu arteritis (TAK), the other most common large vessel vasculitis, was not significantly associated with ICI. In addition, anti-CTLA-4 antibodies were significantly associated with an increased reporting of GCA versus anti-PD-(L)1 monotherapies despite wider use and irAEs reporting of anti-PD-(L)1 versus anti-CTLA-4 in the database (Figure 1C; Table 2). No other statistically significant association with CTLA-4 versus PD-(L)1 blockade was retrieved for the other types of vasculitis associated with ICI in general (Figure 1C; Table 2). Of note, combination therapy versus monotherapies were not significantly different in terms of GCA reporting (Table 2). Together, these results indicate that anti-CTLA-4, but not anti-PD-(L)1, therapy is significantly and specifically associated with GCA, but no other vasculitis, overreporting.

### CTLA-4, but Not PD-1, Is Overexpressed in Aortic Tissue of GCA

To further ascertain a role for CTLA-4 signaling in GCA, we performed immunohistochemistry on paraffin-embedded aorta tissue slides from noninflamed control arteries (CTRL) or GCA and looked for CTLA-4 (n=5 biological replicates) and PD-1 (n=4 biological replicates) expression. We first observed that CTLA-4 was expressed in the whole sections of both CTRL and GCA slides, whereas the PD-1 protein was barely detectable (Figure 2A and 2B). We also observed that the CTLA-4 protein, but not PD-1, was significantly more present in GCA aortic sections versus CTRL (Figure 2B), which is not the case for TAK (data not shown). Importantly, the signals we observed were specific and not artifactual, as matching isotype controls comparatively show low intensities in the tissues (Figure S1A and S1B).

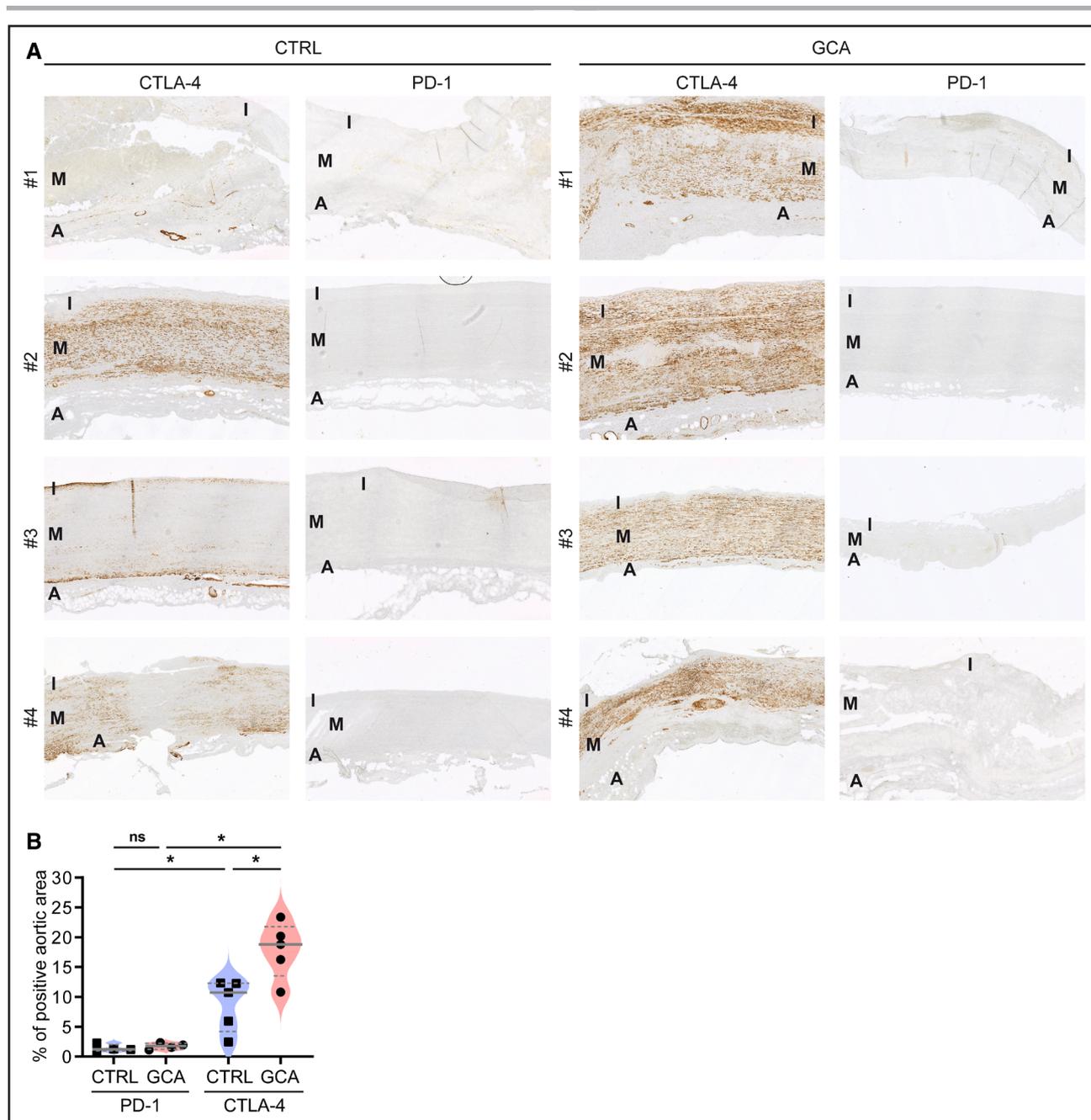
### CD4<sup>+</sup> CTLA-4<sup>+</sup> T-Cell Compartment Is Deeply Altered in GCA Aorta

Given that CTLA-4 is constitutively expressed in the periphery, and especially on CD4<sup>+</sup> Tregs,<sup>22</sup> we further investigated the CD4<sup>+</sup> cell compartment within aortic



**Figure 1. VigiBase analysis of immune checkpoint inhibitor (ICI)-related vasculitis conditions.**

**A**, Reporting of giant cell arteritis (GCA) is associated with ICI and particularly anti-CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) in VigiBase. Information components (ICs) and their 95% credibility interval lower end point (IC<sub>025</sub>) comparing different types of vasculitis reported with ICI vs full database in VigiBase. IC<sub>025</sub> >0 is deemed significant. **B**, Evolution of association of GCA reporting with ICI vs full database over time in VigiBase. **C**, Reporting odds ratios (RORs) and their 95% CI, comparing selected vasculitis previously detected as significant signals using IC<sub>025</sub> by type of ICI: anti-PD-1 (anti-programmed death-1) or anti-PD-L1 (anti-programmed death-ligand 1) (αPD-(L)1) vs anti-CTLA-4 (αCTLA-4) monotherapies. ROR 95% CI lower end >1 is deemed significant. Significant signals are shown in black and nonsignificant ones in gray. Further details about the methodology used here are presented in the [Supplemental Material and Methods](#). ANCA indicates antineutrophil cytoplasmic antibodies; N obs, number of cases of adverse event of interest reported in the ICI subgroup; N reac, number of cases of adverse event of interest reported in the overall database.

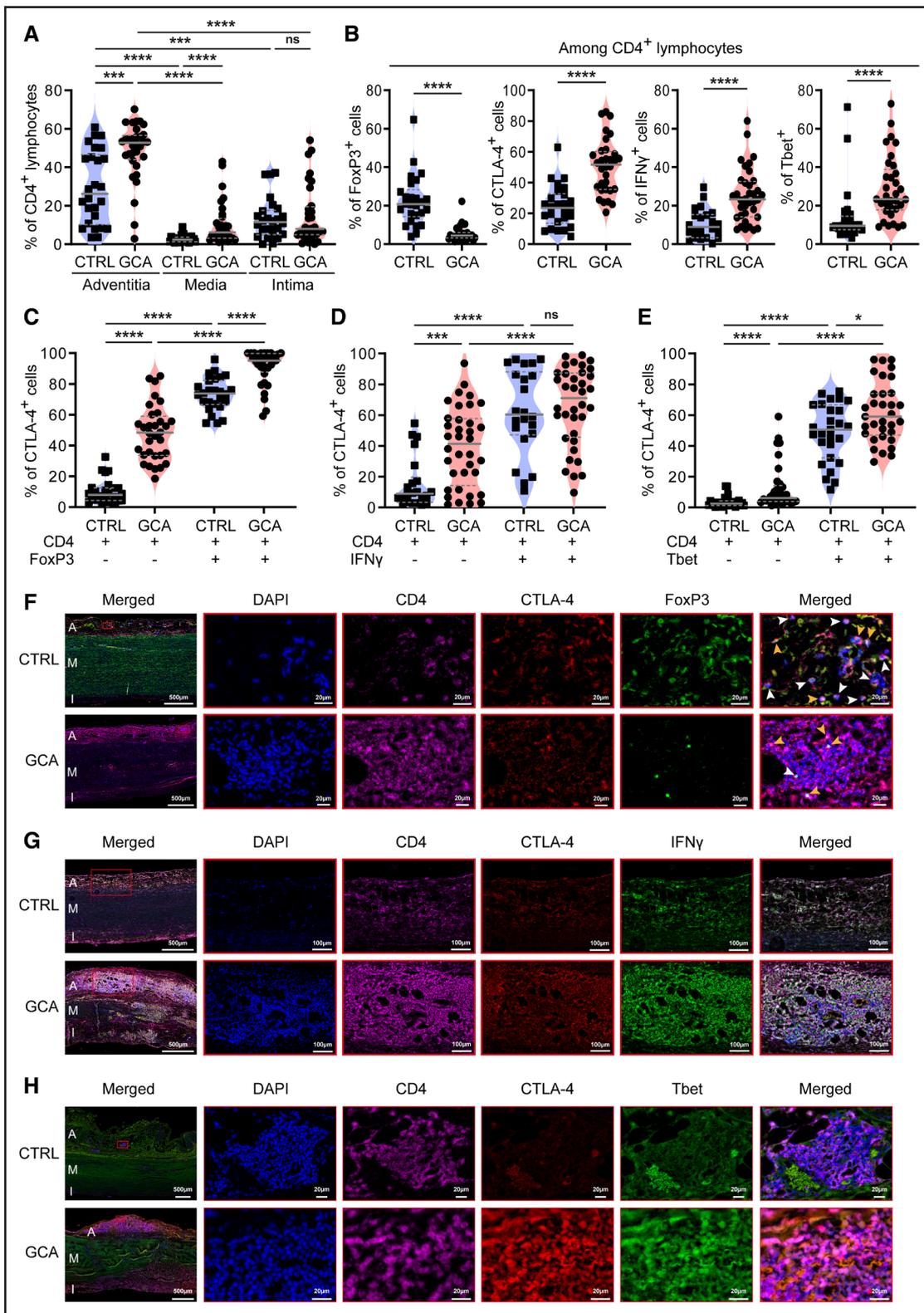


**Figure 2. CTLA-4 (cytotoxic T-lymphocyte-associated protein-4), but not PD-1, is overexpressed in aortic tissue of giant cell arteritis (GCA).**

**A**, Aortic tissue slides from noninflammatory control pathologies (CTRL) or GCA were stained with anti-PD-1 (anti-programmed death-1) ( $n=4$  biological replicates) or anti-CTLA-4 ( $n=5$  biological replicates) immunohistochemistry antibodies before 3,3'-diaminobenzidine tetrahydrochloride (DAB) revelation. **B**, Quantification of the percentage of PD-1<sup>+</sup> or CTLA-4<sup>+</sup> areas was assessed using the Fiji software. Data are displayed as violin plots. A indicates adventitia; I, intima; and M, media.

tissues of GCA. We performed immunofluorescence staining on aorta tissues from either 7 noninflammatory CTRL or 9 GCA patients (biological replicates) using 3 different antibody 4-plexes. Overall, the proportion, absolute number of CD4<sup>+</sup> lymphocytes, and CD4 mean fluorescence intensity were significantly higher in GCA versus CTRL. Most infiltrating CD4<sup>+</sup> lymphocytes were localized in the adventitia tunica (Figure 3A; Figure S2A and S2B). CD4<sup>+</sup> lymphocytes in GCA samples

demonstrated a significantly decreased proportion of FoxP3<sup>+</sup> (forkhead box P3) cells but significantly increased proportion of CTLA-4<sup>+</sup>, IFN $\gamma$ <sup>+</sup> (interferon gamma) and Tbet<sup>+</sup> (T-box expressed in T cells) cells versus CTRL (Figure 3B). Similar results for percentages and absolute numbers were obtained when taking the total adventitia as reference (Figure S2C and S2D). CTLA-4<sup>+</sup> cells were mostly seen along within CD4<sup>+</sup> FoxP3<sup>+</sup> lymphocytes versus CD4<sup>+</sup> FoxP3<sup>-</sup> lymphocytes in CTRL and



**Figure 3. Massive infiltration of adventitia tunica in giant cell arteritis (GCA) aorta deeply alters CTLA-4 (cytotoxic T-lymphocyte-associated protein-4)-expressing cell compartment.** Aorta tissue slides from CTRL (control noninflammatory pathologies) or GCA were antibody stained using three different 4-plex (FoxP3 [forkhead box P3] 4-plex, interferon gamma (IFNγ) 4-plex, and T-box expressed in T cells (Tbet) 4-plex 7 CTRL and 9 GCA biological replicates) or one 8-plex (7 CTRL and 10 GCA biological replicates) and then scanned to reveal fluorescence signal. Full details about the raw data processing are available in the [Supplemental Material and Methods](#). We ended up with 26/22/27/26 CTRL and 32/36/33/52 GCA regions of interest (ROI), considered as independent technical replicates, for the FoxP3 4-plex, IFNγ 4-plex, Tbet 4-plex, and 8-plex, respectively. (Continued)

GCA samples. This difference was even more prominent in GCA versus CTRL (Figure 3C). CTLA-4<sup>+</sup> cells were respectively not significantly increased within CD4<sup>+</sup> IFN $\gamma$ <sup>+</sup> lymphocytes (Figure 3D) but significantly slightly increased within CD4<sup>+</sup> Tbet<sup>+</sup> lymphocytes in GCA versus CTRL (Figure 3E). Of note, we also observed a significantly higher CTLA-4 mean fluorescence intensity among both CD4<sup>+</sup> lymphocytes and total adventitia in GCA versus CTRL (Figure S2E). Figure 3F through 3H shows representative immunofluorescence staining of GCA and CTRL aortas. Of note, we did not observe such important modifications in TAK aortas, which closely resemble CTRL samples regarding the previously mentioned features (data not shown). To further characterize the infiltrating CD4<sup>+</sup> FoxP3<sup>+</sup> lymphocytes, we performed immunofluorescence staining on 7 CTRL and 10 GCA aorta (biological replicates) using an antibody 8-plex that contained CD39 and CD147 markers, 2 surface proteins typically expressed by activated/suppressive Tregs in blood.<sup>23</sup> We observed that among the infiltrating CD4<sup>+</sup> FoxP3<sup>+</sup> lymphocytes in CTRL, the percentage of CD39<sup>+</sup> CD147<sup>+</sup> (double positive [DP]) cells was significantly higher versus CD39<sup>-</sup> CD147<sup>-</sup> (double negative [DN]) cells (Figure S2G, left). These proportions were significantly inverted in GCA, suggesting a less suppressive phenotype of CD4<sup>+</sup> FoxP3<sup>+</sup> cells in GCA versus CTRL. Moreover, these CD4<sup>+</sup> FoxP3<sup>+</sup> DP lymphocytes express significantly more CTLA-4 versus CD4<sup>+</sup> FoxP3<sup>+</sup> DN lymphocytes, both in CTRL and GCA (Figure S2G, right). Interestingly, CD4<sup>+</sup> FoxP3<sup>+</sup> DP (as well as CD4<sup>+</sup> FoxP3<sup>+</sup> DN) cells from GCA express significantly more CTLA-4 versus CTRL. Together, these results indicate that the CD4<sup>+</sup> CTLA-4<sup>+</sup> cell compartment is deeply altered in aorta tissue of GCA. Despite the global and statistically significant upregulation of CTLA-4 within CD4<sup>+</sup> cells together with the decrease of FoxP3<sup>+</sup> cells in GCA, the remaining FoxP3<sup>+</sup> cells express significantly more CTLA-4 versus CTRL.

### Specific Upregulation of CTLA-4–Derived Gene Pathway and Protein in Circulating CD4<sup>+</sup> T Cells in GCA

We hypothesized that similar to what we observed in aorta tissue, circulating CD4<sup>+</sup> T cells in patients with GCA may also be altered. We first analyzed individual expressions of the main immune checkpoints and T-cell costimulatory/coinhibitory signal–associated genes previously reported in literature,<sup>24</sup> as well as related genes

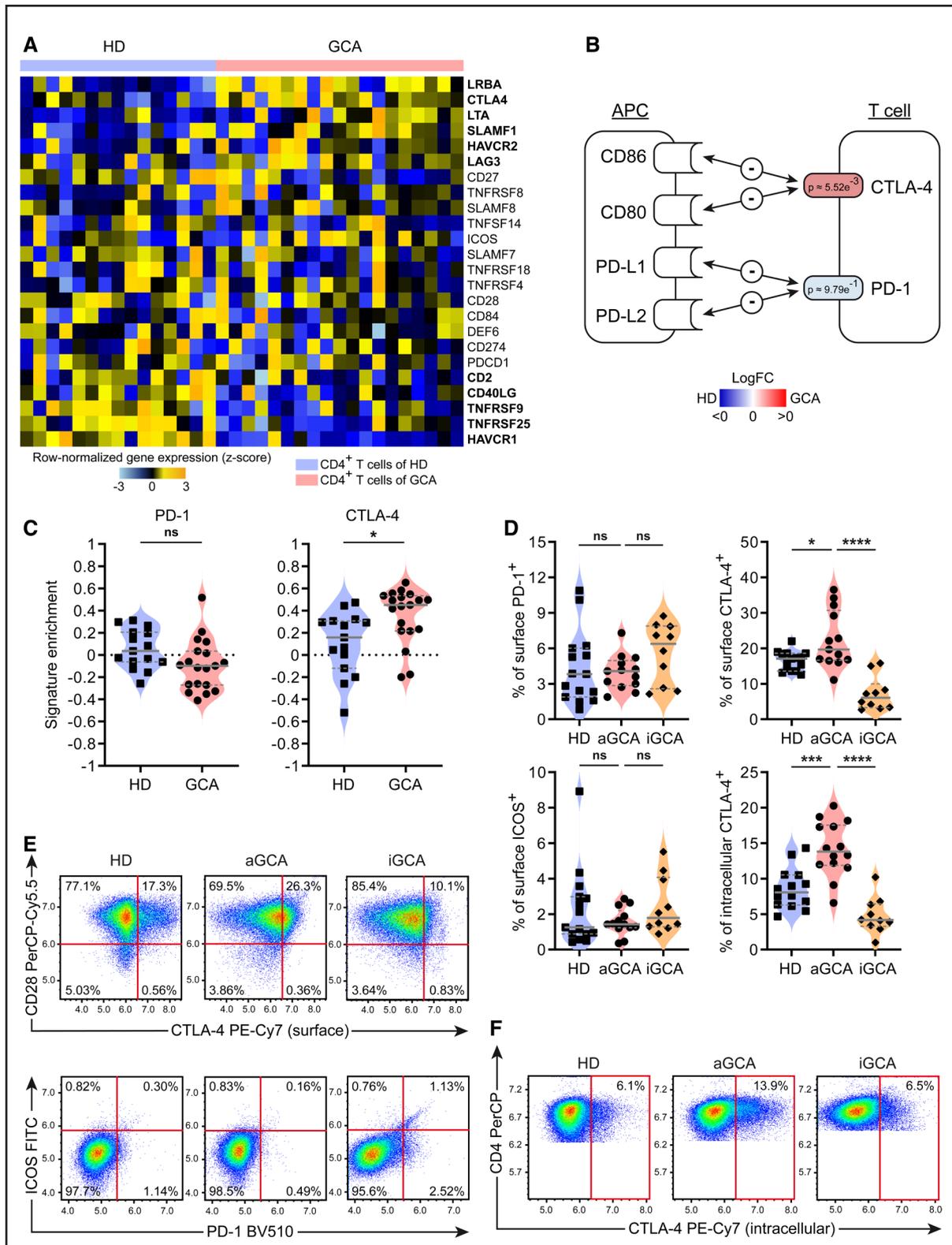
using sorted circulating CD4<sup>+</sup> T-cell transcriptomic data from HD or GCA (Figure 4A). We found that 11 of 24 studied genes were significantly dysregulated between HD and GCA. Interestingly, the *CTLA4* gene was significantly upregulated in GCA, but not *PDCD1*, versus HD (Figure 4B). *CTLA4* gene was the most significantly upregulated coinhibitory signal in GCA CD4<sup>+</sup> T cells. Of note, *LRBA* gene, which codes for a protein essential for the promotion of CTLA-4 intracellular recycling and for the prevention of its degradation,<sup>25</sup> is significantly upregulated in GCA CD4<sup>+</sup> T cells as compared with HD. We obtained similar results with enrichments of gene signatures specific for CTLA-4 and PD-1 pathways generated using published transcriptomic data sets<sup>26,27</sup> (Figure 4C). Flow cytometry analysis of PBMCs confirmed that surface and intracellular CTLA-4 expression on CD4<sup>+</sup> T cells was significantly increased in aGCA versus HD, contrasting with the absence of statistically significant differential expression for surface PD-1 or inducible T-cell Costimulator (ICOS) (Figure 4D through 4F). Interestingly, this CTLA-4 upregulation was not observed anymore (using flow cytometry) in iGCA (Figure 4D through 4F) or in TAK samples (using transcriptome and flow cytometry, data not shown). These results show that CTLA-4 and its related biological pathway, but not PD-1, are specifically and significantly upregulated in circulating CD4<sup>+</sup> T cells of GCA versus HD.

### Despite Being Less Suppressive, Circulating Tregs Overexpress CTLA-4 in GCA

We further dissected the phenotype of CTLA-4–expressing cells within aGCA or iGCA circulating CD4<sup>+</sup> T cells, by staining PBMCs with several Tregs activation and functional markers: CD39, CD147, CTLA-4 (intracellular), Ki-67, Helios, CD304, and CD45RA<sup>23</sup> and then performed a Treg-restricted dimensionality reduction analysis through uniform manifold approximation and projection algorithm.<sup>28</sup>

Similar to what we observed in aortic tissue, we found a significantly decreased proportion of total circulating CD3<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup> CD25<sup>hi</sup> Tregs in aGCA and iGCA versus HD (Figure 5A). Among all the tested markers, we found that CD39 and CD147 allowed to clearly distinguish 2 main Treg subpopulations: CD39<sup>+</sup> CD147<sup>+</sup> (DP) Tregs (plain black surrounding) and CD39<sup>-</sup> CD147<sup>-</sup> (DN) Tregs (dashed black surrounding; Figure 5B; Figure S3A). DP Tregs proportion among total Tregs was significantly decreased in aGCA versus

**Figure 3 Continued.** **A**, Percentages of infiltrating CD4<sup>+</sup> (cluster of differentiation 4) lymphocytes. **B**, Percentages of FoxP3<sup>+</sup>, CTLA-4<sup>+</sup>, IFN $\gamma$ <sup>+</sup>, and Tbet<sup>+</sup> cells among adventitia-infiltrating CD4<sup>+</sup> lymphocytes. **C** through **E**, Percentages of adventitia-infiltrating CTLA-4<sup>+</sup> cells (**C**) among CD4<sup>+</sup> FoxP3<sup>-/+</sup>, (**D**) among CD4<sup>+</sup> IFN $\gamma$ <sup>-/+</sup> lymphocytes, or (**E**) among CD4<sup>+</sup> Tbet<sup>-/+</sup> lymphocytes. **F** through **H**, Representative images for the FoxP3 4-plex, IFN $\gamma$  4-plex, and Tbet 4-plex, respectively. Arrows indicate DAPI<sup>+</sup> CD4<sup>+</sup> FoxP3<sup>+</sup> lymphocytes (white, CTLA-4<sup>-</sup> cells and orange, CTLA-4<sup>+</sup> cells, respectively). These representative images come from samples that both show comparable tissue region/area and most closely match the mean of their associated group of origin. A indicates adventitia; I, intima; and M, media.



**Figure 4. CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) and its specific immune checkpoint signature are significantly upregulated in giant cell arteritis (GCA).**

**A**, Heat map showing MACS-sorted CD4<sup>+</sup> T-cell transcriptomic data from 15 healthy donors (HD) and 19 GCA of main immune checkpoint-related genes. Genes written in bold are significantly dysregulated between HD and GCA. **B**, Schematic representation of *CTLA4* and *PDCD1* gene expression. **C**, Enrichments of gene sets specific for CTLA-4 and PD-1 pathways were calculated using GSVA Bioconductor R package. Violin plots for PD-1 (left) or CTLA-4 (right) pathways are displayed. **D**, Peripheral blood mononuclear cells (PBMCs) from HD, active GCA (aGCA), and inactive GCA (iGCA) were thawed and stained with antibodies against several surface (PD-1, ICOS (Continued)

HD, whereas DN Tregs were significantly increased (Figure 5C). As expected, DP Tregs globally present a more activated/suppressive phenotype versus DN Tregs as they expressed significantly more intracellular CTLA-4, Ki-67, Helios, and CD304, both in percentage (Figure 5D and 5E) and mean fluorescence intensity (Figure S3B and S3C). Strikingly, CTLA-4 was the only marker to be significantly upregulated in Tregs from aGCA versus HD, both in DN and DP Treg compartments. In a more general manner, CTLA-4<sup>+</sup> Tregs were significantly increased in aGCA versus HD (Figure 5F). In fact, the absolute differences in CTLA-4<sup>+</sup> cell frequencies between aGCA and HD were more important for Tregs than Tconv. Interestingly, the previously described phenomena were not observed in iGCA samples, which showed a phenotype and activation profile comparable to HD. We also did not observe statistically significant differences in CTLA-4<sup>+</sup> cell frequencies between TAK samples and HD (data not shown). Together, these results clearly indicate that despite the globally and significantly reduced Tregs proportion observed in GCA, only Tregs from aGCA patients show a decreased activated/suppressive phenotype. Nevertheless, in aGCA, these Tregs still manage to upregulate CTLA-4 specifically and significantly.

### Increased Sensitivity to Ipilimumab-Mediated In Vitro Depletion of CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs in GCA

To better define a functional role for CTLA-4 among CD4<sup>+</sup> T cells in GCA, we depleted dividing Treg/Tconv subpopulations *ex vivo* using ipilimumab. We incubated PBMCs from GCA and HD in culture for 48 hours with PBS or increasing doses of ipilimumab and assessed the percentage of intracellular CTLA-4<sup>+</sup> Ki-67<sup>+</sup> cells among Treg/Tconv subpopulations by flow cytometry. At low ipilimumab concentrations, no statistically significant differences were observed between HD and GCA. Starting from 1 µg/mL ipilimumab concentration, we observed a marked dose-dependent decrease of intracellular CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs for both GCA and HD versus untreated or low-dose ipilimumab (Figure 5G). These depletions were greater for CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Treg subpopulations versus their Tconv equivalent. Notably, at 10 µg/mL, we observed a significantly more pronounced depletion of CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs in GCA versus HD. These *in vitro* results show that activated/proliferating Treg populations in GCA are significantly more sensitive to ipilimumab-mediated *in vitro* depletion versus HD.

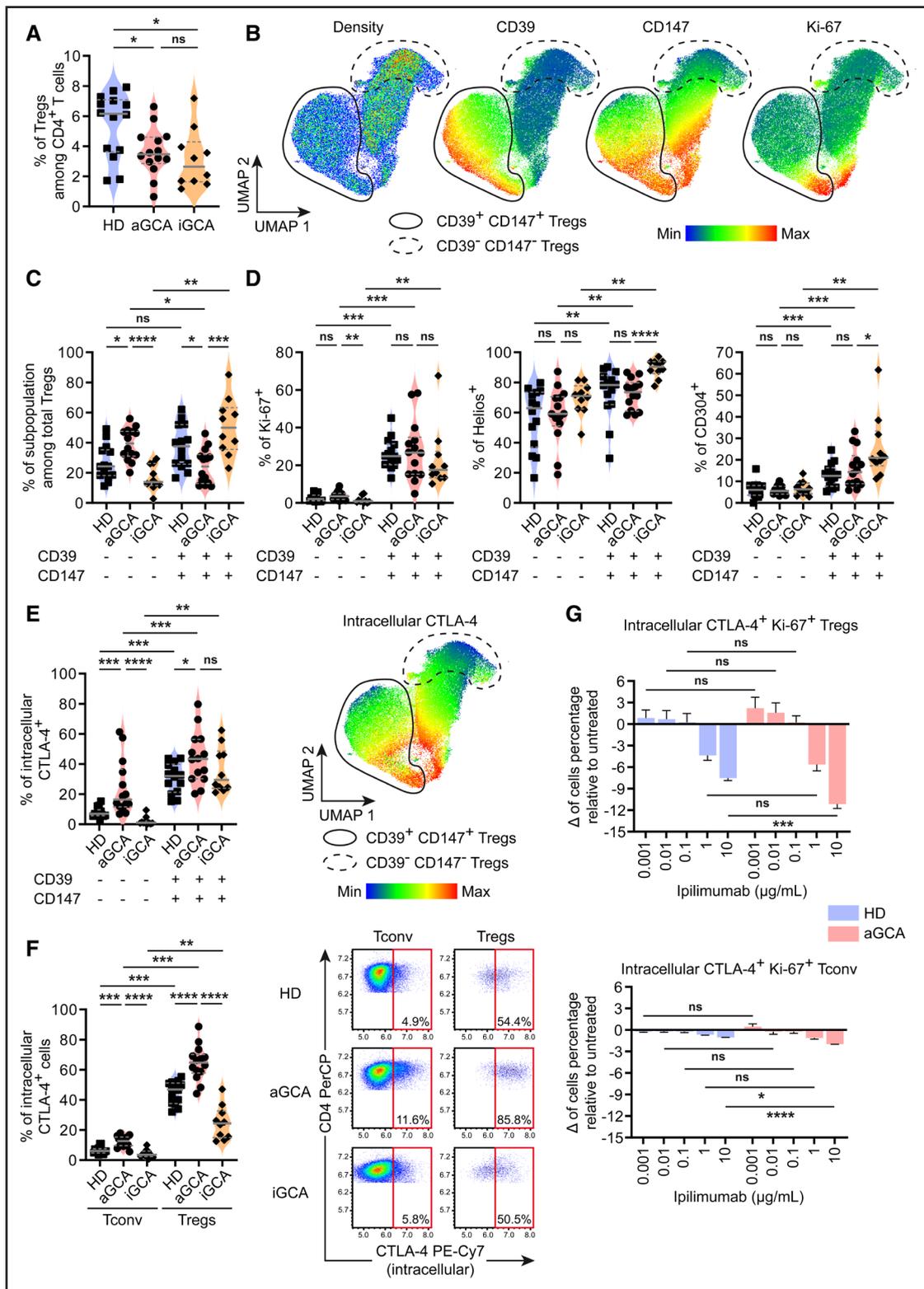
## DISCUSSION

Our translational study suggests that CTLA-4 inhibition by ICI was specifically and significantly associated with overreporting of GCA versus other types of vasculitis. This role of the CTLA-4 pathway was further dissected by several complementary techniques, revealing a critical involvement of CTLA-4<sup>+</sup> Tregs in GCA pathophysiology, both on the aortic tissue and circulation. Our results additionally show that despite a significantly reduced number of Tregs in GCA coupled to their less suppressive phenotype versus controls, Tregs still managed to upregulate CTLA-4 specifically and significantly. Together, our results suggest that strategies aiming at modulating the CTLA-4 signaling pathway in patients with GCA might prove beneficial.

Anti-CTLA-4 and anti-PD-(L)1 therapies used in patients with cancer can induce distinct patterns of irAEs, suggesting their divergent roles in the maintenance of self-tolerance, depending on the organs.<sup>16,29</sup> Overall, the prevalence and severity of irAEs is greater with anti-CTLA-4 drugs or combination of ICI including an anti-CTLA-4 as compared with anti-PD-(L)1 monotherapies.<sup>16,17</sup> In fact, the combination of anti-CTLA-4 and anti-PD-(L)1 treatments seems to globally exacerbate the frequency and the severity of irAEs.<sup>17</sup> Despite the fact that, in humans, severe cardiovascular system morbidities were evidenced following ICI,<sup>16,30</sup> only a few types of vasculitis were significantly and specifically overreported in our study. Among them, GCA was the disease that was the most disproportionately associated with ICI treatments, actually showing a clear skew toward anti-CTLA-4 versus anti-PD-(L)1 monotherapies. Moreover, several studies evaluating ICI effectively reported patients with metastatic melanoma treated by ipilimumab in whom GCA subsequently developed.<sup>18,19</sup> According to these studies, ipilimumab-induced GCA seems to resemble spontaneous forms of the disease as they share common clinical and histological features.

Conversely, in mice, autoimmune manifestations following the *Ctla4* gene deletion are better described and appear to be age dependent: while constitutive *Ctla4*<sup>-/-</sup> mice all die within 30 days of age because of a massive and multiorgan lymphoproliferative syndrome,<sup>31,32</sup> temporal *Ctla4* deletion in adult mice induces less severe autoimmune manifestations.<sup>32</sup> Furthermore, recent work by Wei et al<sup>33</sup> pointed out that heterozygous loss of *Ctla4* in mice in the context of associated homozygous *Pdcd1* deletion leads to about 50% mortality rate due to severe cardiovascular issues, whereas all mice remain healthy

**Figure 4 Continued.** [inducible T-cell COStimulator], and CTLA-4, 15 HD, 13 aGCA, and 10 iGCA) or intracellular (CTLA-4, 14 HD, 14 aGCA, and 10 iGCA) immune checkpoints. CD4<sup>+</sup> (cluster of differentiation 4) T-cell restricted data are displayed using violin plots. **E** and **F**, Representative pseudocolor dot plots depict the expression of **(E)** surface CTLA-4, PD-1, CD28 (cluster of differentiation 28), and ICOS or **(F)** intracellular CTLA-4 by CD4<sup>+</sup> T cells from HD, aGCA, and iGCA PBMCs. These representative dot plots come from samples that both show similar cellularity and most closely match the mean of their associated group of origin.



**Figure 5. Blood regulatory T cell (Treg) compartment and associated CTLA-4 (cytotoxic T-lymphocyte–associated protein-4) expression are deeply altered in giant cell arteritis (GCA)**

Peripheral blood mononuclear cells (PBMCs) from 14 healthy donors (HD), 14 active GCA (aGCA), and 10 inactive GCA (iGCA) samples were thawed and antibody stained before flow cytometry revelation. **A**, Violin plot showing the percentage of FoxP3<sup>+</sup> (forkhead box P3) CD25<sup>hi</sup> cells (Tregs) among total CD4<sup>+</sup> T cells. **B**, Dot plots showing the resultant 2-dimensional UMAP parameters for GCA samples, individually overlaid with CD39, CD147, and Ki-67 markers. CD39<sup>-</sup> CD147<sup>-</sup> (double negative [DN]) and CD39<sup>+</sup> CD147<sup>+</sup> (double positive [DP]) Tregs were surrounded in plain and dashed black, respectively. **C**, Violin plot showing the percentage of DN and DP cells among total Tregs in HD, aGCA, or iGCA. **D** and **E**, Violin plots showing the frequencies of (D) Ki-67<sup>+</sup>, Helios<sup>+</sup>, and CD304<sup>+</sup> or (E, left) CTLA-4<sup>+</sup> cells among DN or DP (Continued)

when at least 1 copy of the *Pdcd1* gene was present. This phenotype is consistent with fulminant myocarditis cases reported in metastatic melanoma patients treated with ipilimumab and nivolumab ICI combination.<sup>34</sup> Together, these results clearly suggest that CTLA-4–associated, PD-1–associated,<sup>20</sup> and potentially other immune checkpoint–associated pathways may contribute and synergize to trigger and maintain immune system activation in GCA.

Herein, we demonstrated that CTLA-4 is specifically upregulated on CD4<sup>+</sup> T cells, both in blood and aorta, in GCA versus controls. CTLA-4 is mainly expressed by T cells, although its distribution varies considerably: whereas it is constitutively expressed by Tregs,<sup>22</sup> it is only upregulated after prolonged activation on conventional/effector T cells.<sup>35,36</sup> We highlighted in GCA patients that CTLA-4 expression by Tregs was even more prominent versus controls or Tconv, both in blood and aorta. As CTLA-4 binds CD80/CD86 (expressed by antigen-presenting cells) with a much higher affinity than CD28,<sup>35</sup> a deeper understanding of the role of these signals in the GCA context appears to be critical. In an effort to better understand how this axis in GCA could be shaped, we also sorted CD19<sup>+</sup> circulating B cells from 6 HD and 6 patients with GCA and analyzed their transcriptome. We observed that *CD80* but not *CD86* gene expression was significantly upregulated in GCA versus HD. Consistently, a recent literature study highlighted a role of B cells in GCA by demonstrating their massive infiltration, activation, and self-organization into tertiary lymphoid structures within aorta.<sup>37</sup> Together, these data may provide an insight into why blocking T-cell costimulation with abatacept (ie, CTLA4 Ig fusion protein) led to significantly reduced relapse risk of GCA at 12 months.<sup>38</sup>

The precise mechanisms by which ipilimumab could induce irAEs are still poorly understood but have been hypothesized to involve a breakdown of peripheral tolerance and induction of organ-specific inflammatory processes.<sup>17,19</sup> In controls, we observed, both in blood and aorta, that activated/suppressive DP Tregs are present in a significantly greater proportion than poorly suppressive DN Tregs. In aGCA, we instead significantly quantified that (1) Tregs are less numerous, (2) Tregs are less globally suppressive, and (3) Treg subpopulations remaining in aGCA express specifically more CTLA-4 versus control. Noteworthily, our findings in blood and aorta fit well with a recent report showing that GCA Tregs are dysfunctional and present less suppressive/inhibitory

capabilities.<sup>39</sup> One can hypothesize that the few remaining Tregs in aGCA upregulate CTLA-4 as a compensatory mechanism, trying to retain their functionality. As CTLA-4 is frequently coexpressed with Ki-67, this potential mechanism may explain the high sensitivity of circulating CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Tregs to ipilimumab-mediated in vitro depletion in aGCA versus HD. Along with their ability to deeply impair Tregs function and survival in cancer, ICIs are also known to concomitantly increase Th1/Th17 cell responses and associated cytokine secretion.<sup>40–42</sup> Conversely, lesional T cells in GCA produce multiple effector cytokines and especially IFN $\gamma$ , IL (interleukin)-17, and IL-21,<sup>3,4,43</sup> accompanied by a decreased proportion of Tregs,<sup>4</sup> which is indicative of a general hyperresponsiveness. We are conscious that our study lacks a deeper in vitro study of GCA Tregs, but their low abundance within GCA PBMCs did not allow us to have enough cellularity to correctly put them in culture. Furthermore, we could have also used existing mice models of vasculitis, yet not totally physiologically relevant, to reproduce our results.

In conclusion, our results highlighted the instrumental role of CTLA-4 immune checkpoint in GCA based on a translational study from a pharmacovigilance database to a deep immunological and mechanistic analysis. Our observations also imply that modulating CTLA-4 signaling pathway might be promising in the management of GCA.

## ARTICLE INFORMATION

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**Figure 5 Continued.** Tregs in HD and GCA. A dot plot showing UMAP parameters for GCA Tregs overlaid with CTLA-4 marker is shown in **E (right)**. **F, Left**, Violin plot showing the percentage of CTLA-4<sup>+</sup> cells among conventional T cell (Tconv) or Treg compartments in HD, aGCA, and iGCA. Representative pseudocolor dot plots for CTLA-4 expression are shown in **F (right)**. These representative dot plots come from samples that both show similar cellularity and most closely match the mean of their associated group of origin. **G**, PBMCs from 12 HD or 12 aGCA samples were thawed and put in culture in 96 well plates during 48 hours together with plate-bound anti-CD3/anti-CD28 and PBS or increasing doses of ipilimumab. Afterward, cells were recovered and analyzed by flow cytometry, where CTLA-4<sup>+</sup> Ki-67<sup>+</sup> Treg and Tconv frequencies were measured. Data are normalized groupwise and population-wise using their ipilimumab-untreated respective control. Data are displayed as bar plots showing mean $\pm$ SEM.

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### Disclosures

None.

### Supplemental Material

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