JCI The Journal of Clinical Investigation

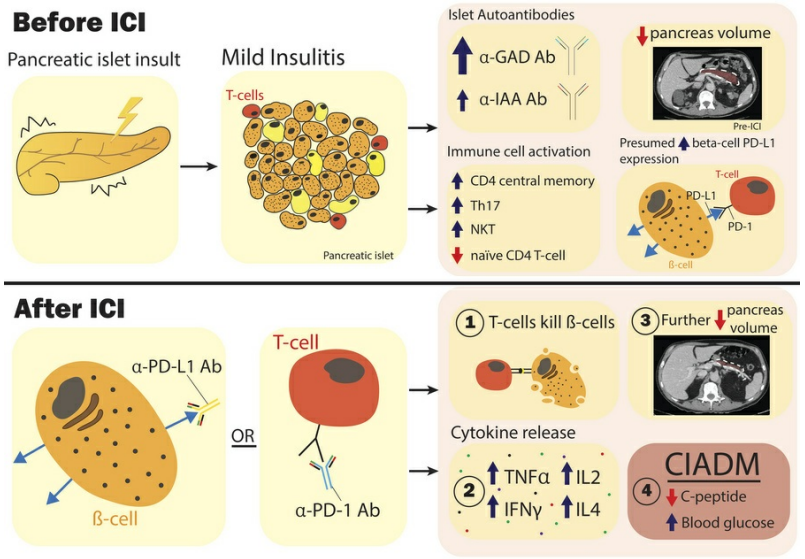
Pancreatic volume and immune biomarkers predict checkpoint inhibitor-associated autoimmune diabetes in humans

Linda Wu, John M. Wentworth, Christopher Liddle, Nicole Fewings, Matteo Carlino, David A. Brown, Roderick Clifton-Bligh, Georgina V. Long, Richard A. Scolyer, Nicholas Norris, Sarah C. Sasson, Venessa H.M. Tsang, Alexander M. Menzies, Jenny E. Gunton

J Clin Invest. 2025. https://doi.org/10.1172/JCI192938.

Clinical Research and Public Health | In-Press Preview | Autoimmunity | Endocrinology | Oncology

Graphical abstract



Title: Pancreatic volume and immune biomarkers predict checkpoint inhibitor-associated autoimmune diabetes in humans

Short Title: Predicting checkpoint inhibitor diabetes

Authors: Wu Linda [1-4], Wentworth John M [5], Liddle Christopher [4], Fewings Nicole [1], Carlino Matteo [2,6], Brown David A [1,2,4,7], Clifton-Bligh Roderick [3,4], Long Georgina V [3,4,6], Scolyer Richard A [4,6], Norris Nicholas [1,4], Sasson Sarah C [2,4,7], Tsang Venessa HM [3,4], Menzies Alexander M [3,4,6], Gunton Jenny E [1,2,4]

Affiliations:

[1] Centre for Diabetes, Obesity and Endocrinology Research, Westmead Institute for Medical Research, Westmead, Sydney, NSW. Australia.
[2] Department of Diabetes and Endocrinology, Westmead Hospital, NSW. Sydney. Australia.
[3] Department of Diabetes and Endocrinology, Royal North Shore Hospital, NSW. Sydney. Australia.
[4] Westmead Institute for Medical Research, University of Sydney, NSW. Australia.
[5] Department of Diabetes and Endocrinology Royal Melbourne Hospital and Walter and Eliza Hall Institute of Medical Research, Melbourne. Australia.
[6]Melanoma Institute Australia, Sydney, NSW. Australia.
[7] NSW Health Pathology, Westmead. Sydney. NSW. Australia.

Corresponding author:
Prof Jenny Gunton,
Room 2040, Level 2, Westmead Hospital. Sydney. NSW. Australia. 2145.
Jenny.gunton@sydney.edu.au
Tel +61 2 8890 8089

Word count: 4657 (introduction to acknowledgement)

Keywords: Diabetes; Immune Checkpoint Inhibitors; Autoimmunity; Type 1 diabetes;

Melanoma; Cancer

Abbreviations:

CCL = C-C motif chemokine ligand

CIADM = Checkpoint inhibitor associated autoimmune diabetes

CT = Computed tomography

CXCL = C-X-C motif chemokine ligand

FDR = false discovery rate

GAD = glutamic acid decarboxylase

HLA = human leukocyte antigen

IAA = islet autoantigen

ICI = Immune checkpoint inhibitor

IL = interleukin

IRAE = immune-related adverse event

PBMC = peripheral blood mononuclear cell

PD-1 = programmed cell death protein 1

PD-L1 = programmed cell death ligand 1

T1D = Type 1 diabetes

TGF = Transforming growth factor

TNF = tumour necrosis factor

ABSTRACT

Background Checkpoint inhibitor-associated autoimmune diabetes (CIADM) is a rare but life-altering complication of immune checkpoint inhibitor (ICI) therapy. Biomarkers that predict type 1 diabetes (T1D) are unreliable for CIADM.

Aim

To identify biomarkers for prediction of CIADM.

Methods

From our prospective biobank, 14 CIADM patients who had metastatic melanoma treated with anti-PD-1 ± anti-CTLA4 were identified. Controls were selected from the same biobank, matched 2:1. Pre-treatment, on-ICI and post-CIADM serum and peripheral blood mononuclear cells (PBMCs) were analysed. Serum was analysed for T1D autoantibodies, C-peptide, glucose and cytokines. PBMCs were profiled using flow cytometry. Pancreatic volume was measured using CT volumetry.

Results

Before treatment, CIADM patients had smaller pancreatic volume (27% reduction, p=0.044) and higher anti-GAD antibody titres (median 2.9 versus 0, p=0.01). They had significantly higher baseline proportions of Th17 helper cells (p=0.03), higher CD4+ central memory cells (p=0.04) and lower naïve CD4+ cells (p=0.01). With ICI treatment, greater declines in pancreatic volume were seen in CIADM patients (p<0.0001). Activated CD4+ subsets increased significantly in CIADM and controls with immune-related adverse effects (IRAE) but not controls without IRAE.

Using only pre-treatment results, pancreatic volume, anti-GAD antibody titre and baseline immune flow profile were highly predictive of CIADM development, with an area under the curve (AUC) of >0.96.

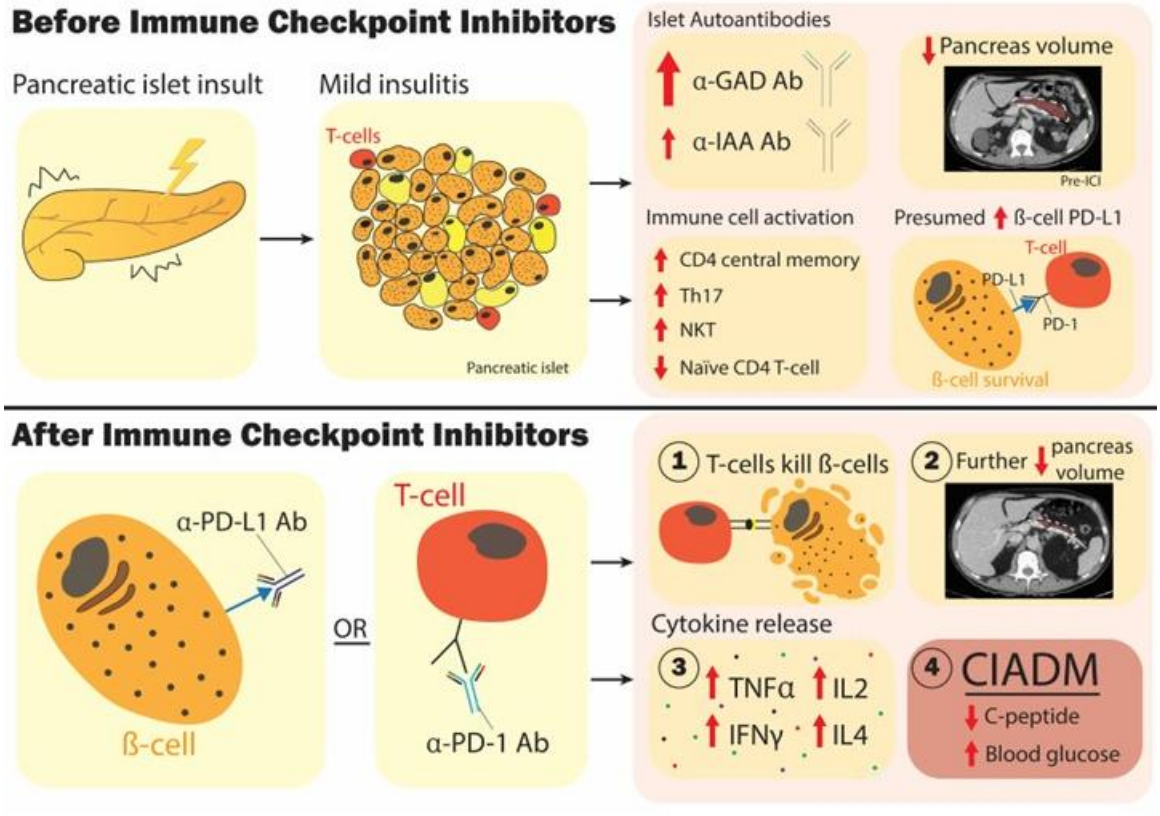
Conclusions

People who develop CIADM are immunologically predisposed and have antecedent pancreatic and immunological changes that accurately predict disease with excellent sensitivity. These biomarkers could be used to guide ICI use, particularly when planning treatment for low-risk tumours.

Funding

JEG is supported by NHMRC Investigator grant 2033228. AMM by NHMRC Investigator grant 2009476 and GVL by NHMRC Investigator grant 2007839.

Graphical Abstract



Introduction

Immune checkpoint inhibitors (ICIs) have transformed the treatment for many malignancies since their initial introduction in melanoma therapy. Eleven ICIs are now FDA-approved for at least 43 indications in a wide range of malignancies (1). Whilst primarily used in the setting of metastatic cancer, recent studies also demonstrate benefits in the adjuvant and neoadjuvant settings (2, 3).

As use of ICIs increases, the corresponding incidence of immune-related adverse effects will also rise. Amongst these, checkpoint inhibitor related autoimmune diabetes mellitus (CIADM; also termed ICI-DM) is of particular interest due to the major, life-long physical and psychosocial impacts of insulin requiring diabetes and the propensity for fulminant onset with high risk of diabetic ketoacidosis. We have previously demonstrated that CIADM bears similarities to its *de novo* counterpart type 1 diabetes (T1D) with respect to insulin deficiency and lifelong insulin dependency. However, there are also distinct differences including a high prevalence of T1D antibody negativity and fulminant beta cell failure, thus warranting separate diagnostic criteria and evaluation (4). CIADM develops in 0.4-1.9% of people treated with therapies directed against programmed death 1 (PD1) or programmed death ligand 1 (PDL1) (5–9).

The ability to estimate an individual's risk of developing serious immune-related adverse effects prior to starting ICI would inform treatment decisions, especially in the adjuvant setting and where effective alternative treatments are available. Studies show that the overall risk of any immune-related adverse effects is associated with higher baseline CD4+ counts (10), early T regulatory cell expansion (11), increased CD8+ clonal responses (12),

more diverse T cell repertoire (13), higher cytokine levels at baseline and early in treatment (11, 14, 15), neutrophil to lymphocyte ratio (16) and genetic variants (17).

In T1D, anti-islet autoantibodies predict risk of disease with high accuracy (18). HLA (human leukocyte antigen) haplotypes are also strongly linked to T1D risk with ~95% of people having high-risk HLA DR3 and / or DR4, and genetic risk scores are available to further delineate risk (19). A decline in pancreatic volume is associated with risk of progression from pre-clinical to overt T1D (20). Flow cytometry shows differences in CD4+ T follicular helper cells, T regulatory cells, naïve and Th17 cell subsets associated with T1D onset (21–25). Islet-specific autoreactive T cells are a promising T1D biomarker but assays are subject to HLA type restrictions (26, 27).

This aim of this study is to identify potential biomarkers for CIADM risk prior to commencement of ICI therapy and early during treatment. A secondary aim is to identify biomarkers for risk prediction after ICI-commencement but before CIADM onset. We compare CIADM cases to controls receiving ICI pre-treatment, early during treatment and after CIADM diagnosis.

Results

A summary of the methods is shown in Figure 1 and the flow cytometry gating strategy is shown in Supplementary Figure 1. Fourteen patients with CIADM and 28 matched controls treated with ICI were included. All patients had metastatic melanoma. Of the total samples sought, 2 PBMC samples were not available for CIADM patients at the pre-treatment timepoint. Baseline characteristics are shown in Table 1. Prior exposure to other anti-cancer treatment was predominantly dabrafenib and trametinib therapy.

Subclinical anti-GAD and anti-IAA antibody levels are associated with CIADM

Before ICI-treatment, glutamic acid decarboxylase auto-antibody (Anti-GAD) titres were significantly higher in CIADM cases than controls (Figure 2A, p=0.002, Mann-Whitney). Despite the higher antibody levels with this sensitive assay, only 2 patients (14%) had levels above the reference range for anti-GAD before ICI exposure, with 1 on but not above the top of the reference range (dotted line) and an additional 6 patients having a level above the threshold of detection (dashed line), giving a total of 64% of CIADM patients having detectable levels compared to 4 ICI treated controls who did not develop diabetes (14%, $p<0.001$ vs CIADM patients, Chi-square with Yates correction).

Figure 2B shows that anti-insulin autoantibodies (IAA) titres were also significantly higher in pre-treatment CIADM patients than in pre-treatment controls (p=0.048, Mann-Whitney). Seven CIADM patients were above the threshold of detection for the assay (54%) compared to 6 of 28 controls (21%, p=0.038). As insulin exposure is known to provoke IAA development, it should be noted that no patients had exposure to insulin prior to ICI

treatment. However, the rise of IAA seen in CIADM patients after diagnosis and insulin treatment is consistent with this also being common after diagnosis of type 1 diabetes.

Autoantibody positivity above the clinical test threshold was not significantly associated with increased risk of presentation with diabetic ketoacidosis or earlier with CIADM diagnosis.

Pancreatic volume is lower in CIADM patients before ICI treatment

Pancreatic volume was measured using computed tomography (CT) scans. Pancreatic volume on CT scans was lower before ICI exposure in people who went on to develop CIADM than in controls (median 60 versus 73mls, Figure 2C, p=0.019). All but one patient who went on to develop CIADM had baseline pancreatic volume <75mls (92%) compared to 14 of 28 controls (50%, p=0.0007 by Chi-Square with Yates correction).

Antibody levels and pancreatic volume change with ICI treatment

After ICI exposure, anti-GAD titres were significantly higher in people who went on develop CIADM than in on-treatment controls (p=0.008, Kruskall-Wallis with Dunn's correction for multiple comparisons, Figure 2D). Anti-IAA titres tended to be higher on-ICI in those who developed CIADM than controls on-ICI, but this did not remain significant after correction for multiple comparisons (p=0.09, Figure 2E).

Figure 2F shows that anti-insulinoma antigen 2 (IA2) titres were higher on-ICI in CIADM patients than in on-ICI controls (p=0.045). In Figure 2G, anti-zinc transporter 8 (ZnT8) titres were higher at CIADM-diagnosis than in on-ICI controls (p=0.04) but did not differ before diabetes-onset.

Pancreatic volume was profoundly reduced at CIADM diagnosis compared to before-ICI ($p<0.01$) and was substantially lower than controls on-ICI ($p<0.0001$, Figure 2H). Most CIADM patients did not have on-treatment scan prior to onset of CIADM.

Pre-treatment and pre-diabetes glucose and C-peptide levels do not predict future CIADM

Insulin secretion was assessed by measuring C-peptide and concurrent glucose. People who went on to develop CIADM did not have lower C-peptide before ICI treatment, or on-ICI before CIADM (Figure 2I).

C-peptide fell from a median of 1.0 (IQR 0.6-1.4) nmol/L pre-ICI and 1.1 (0.6-1.8) on-ICI to 0.05 (0-0.3) nmol/L post diagnosis for CIADM patients. In controls it remained normal (Figure 2I).

Formal blood glucose was not available for all CIADM patients after diagnosis, before commencement of insulin, and the available glucose levels did not differ significantly (Figure 2J). Overall, neither C-peptide nor serum glucose are predictive for future CIADM.

Changes in antibody titres with ICI-treatment within individuals were examined to assess whether this may be an independent predictor of CIADM (Figure 3). No pattern of antibody change during treatment significantly predicted CIADM.

Altered circulating cytokine levels are associated with CIADM

Figure 4 depicts circulating cytokine concentrations at the different timepoints in CIADM patients and controls. No cytokines showed differential expression before ICI therapy. Interferon-γ (IFNγ), interleukin 1β (IL-1β), and tumor necrosis factor-α (TNFα) are the cytokines most classically associated with T1D. IFNγ was elevated at CIADM diagnosis

compared to before or on-ICI (Figure 4A, $p<0.05$). IL-1β did not show any significant differences (Figure 4B). Figure 4C shows that TNFα also rose significantly at the time of CIADM diagnosis compared to baseline or to on-ICI in CIADM patients.

Interleukin 2 (IL-2) and IL-4 were both also significantly higher at CIADM diagnosis than at baseline (Figures 4D and 4E, $p<0.05$). Interleukins 6, 8 (also called CXCL8), 10, 12, and 17A did not differ between groups (Figures 4E-J), nor did CCL2 (chemokine C-C motif ligand 2), free TGFβ (transforming growth factor) or C-X-C motif chemokine ligand 10 (CXCL10), (Figures 4K-M).

Cytokine levels predict immune related adverse effects (IRAE)

The 9 controls who did not develop any known immune related adverse event were compared to people who developed CIADM plus controls who developed an IRAE to test whether circulating cytokine levels may be predictive of developing any IRAE (Figure 5). When compared to controls without IRAE, before ICI treatment, IRAE patients had significantly higher levels of IL-2, IL-6, IL-17A, CCL2, and free TGFβ before commencing ICI therapy (all $p<0.05$; Figures 5E, 5F, 5J, 55K and 5L, all Kruskall-Wallis with Dunn's correction for multiple comparisons).

IRAE patients after ICI exposure had higher IL-6 (Figure 5F), IL-17A (Figure 5J) and CXCL10 (Figure 5M) than on-ICI levels in people with no IRAE.

People who developed CIADM have a more activated immune system at baseline

Immuno-phenotyping of circulating PBMCs reveals significant differences at baseline between patients who developed CIADM and controls. CIADM patients, before ICI-exposure,

had fewer naïve CD4+ T-cells (Figure 6A, $p<0.05$), more Th17 cells (Figure 6B, $p=0.001$) and more CD4+ central memory cells (CM, $p<0.01$, Figure 6C). Pre-ICI CIADM patients also had fewer activated CD8+ CD38+ HLADR+ T cells (Figure 6D, $p<0.05$).

Interestingly, given the fulminant phenotype of diabetes in many CIADM patients, there were also differences in baseline natural killer (NK) cells, with more CD56hi NK cells (Figure 6E, $p<0.01$). These are an NK cell subtype more strongly associated with cytokine and chemokine production (Figure 6F, $p<0.05$).

After ICI treatment, there were no further significant changes in these cells (Figures 6G-L). Other cell subsets including Treg cells were not significantly altered (Figure 6). Figure 8 shows other flow cytometry results for cell types which were not significantly altered.

Immune cell phenotypes also differ with IRAE

Flow cytometry parameters were compared in people with IRAE were compared to those with no IRAE (Figure 88). At baseline, people who went on to develop an IRAE also had fewer naïve CD4 cells at baseline, more Th17 cells, and more CD4+ central memory cells.

After ICI treatment, people with IRAE showed increased CD8+ CD38+ HLADR+ cells (Figure 8D).

Differential gene expression in CD8+ T cells in CIADM

CD8+ T-cells are thought to be the major mediator of beta-cell death in T1D. Circulating CD8+ T-cells were collected and RNA expression was profiled with RNA-sequencing. Surprisingly, before ICI therapy, there were no differentially expressed genes that passed a false discovery rate (FDR) of <0.05 comparing CIADM and control patients. Comparing on-ICI controls to on-ICI CIADM patients pre diagnosis, only 2 genes passed FDR; RNF220 and BCR

(both p=0.044). Comparing on-ICI controls to after-diagnosis CIADM patients, no genes passed FDR testing. This data will be available at GEO INSERT HERE – currently cannot upload with the shutdown.

Receiver Operated Characteristic (ROC) curve analyses of key predictors

The baseline variables that were significantly associated with CIADM development (anti-GAD, anti-IAA, pancreatic volume, CD4+ central memory, CD4+ naïve, Th17 cells, CD8+ HLA-DR+CD38+, and NK CD56hi) are shown in Supplementary Table 1. They were combined in a multiple logistic regression model. Figure 9 shows that this gave a receiver operated characteristic (ROC) curve with an area under the curve (AUC) of 0.968 (95% CI 0.919-1.0, p<0.0001). This was associated with positive predictive value of 92.6% and a negative predictive value of 90.91%.

The data were separately analysed using only antibodies and pancreatic volume, as clinical flow-cytometry testing may not be available in all centres in a clinically meaningful timeframe. Including only anti-GAD, anti-IAA and pancreatic volume in the model gave an ROC curve with AUC of 0.891 (p=0.0001), with negative predictive value of 77.8% and positive predictive value of 82.8%.

Discussion

Here we report a number of baseline predictors of CIADM. Using serial samples, patients and controls were evaluated using a combination of flow cytometry, cytokine expression, autoantibody analysis, RNA-Seq and CT imaging analysis. We identify that CIADM patients have higher levels of anti-GAD and anti-IAA at baseline and lower baseline pancreatic

volume compared to matched controls. CIADM patients had higher baseline Th17+, higher CD4+ central memory cells and lower naïve CD4+ cells than controls. CIADM patients also exhibited differences in lymphocyte expansion early on treatment with higher activated CD4+ CD38+ HLA-DR+ subsets and lower naïve CD4+ subsets compared to controls.

In humans, limited data are available regarding the immunophenotype of CIADM. Hughes *et al* reported a case series of five patients with CIADM and amongst the four patients that had HLA-A2+ haplotyping, two had increased diabetes antigen specific T cells, which were predominantly effector or memory cells (28). A mass cytometry based study of 28 patients with melanoma treated with ICI included two patients with new onset T1DM, which we would term CIADM. This study identified higher activated CD4+ cells in those with severe IRAE of all types on treatment similar to our study, but conversely to our findings found higher naïve CD4+ T cells to be associated with more severe IRAE (29). However, of the two CIADM patients included, no significant differences in comparison to controls were found.

When looking at IRAE studies in general, Lozano *et al's* study of T cell phenotyping in patients treated with ICIs for melanoma, using single cell RNA-Seq revealed baseline and early on treatment expansion in CD4+ T effector memory subsets to be associated with severe IRAE of all types, whereas in our study CD4+ central memory subsets defined by flow cytometry had the strongest association with CIADM at baseline (30). Bukhari *et al* previously identified on single cell sequencing of PBMCs from patients with thyroiditis was associated with higher baseline Th17 subsets (31). Kim *et al* similarly found higher baseline Th17 subsets to be associated with development of severe immune-related adverse effects

of all types in a cohort of patients treated for non-small cell lung cancer and thymic epithelial tumours with ICIs (32).

In type 1 diabetes, both Th1 and Th17 pathways are acknowledged as direct drivers of disease pathogenesis in human and animal studies (33–35). A recent study found that Ustekinumab which binds IL-12 and IL-23 to target Th1 and Th17 cells was able to preserve pancreatic β-cell function in adolescents with recent onset type 1 diabetes (36). We find increased baseline Th17 cell numbers in CIADM patients and associated significant increases of cytokines associated with the Th17 pathway including IL-6, TGFβ, TNF-α and IFN-δ in CIADM patients compared to controls. Interestingly, the majority of changes in circulating immune cells were in CD4+ cells. Consistent with this, there were essentially no changes in gene expression in circulating CD8+ T cells in CIADM patients. The lack of changes in circulating CD8+ T cells is surprising and suggests either that CD8+ cells are not important in CIADM, or more probably that the cells of relevance were not in circulation. In T1D, the pathogenic CD8+ T cells are highly concentrated in the pancreas and pancreatic lymph node (37).

In comparison to other IRAE, one of the unique aspects of CIADM is that its *de novo* counterpart T1D has well established biomarkers in the form of islet autoantibodies, especially anti-GAD, anti-IA2, anti-IAA and anti-ZnT8. It is known that CIADM patients at diagnosis have lower prevalence of those autoantibodies than in T1D (4). We and others have reported pre-treatment anti-GAD positivity in a small proportion of CIADM patients but this has not been extensively tested or compared with controls (38–41). Anti-GAD positivity is present in a small proportion of the general population, with a median specificity of anti-

GAD in the Islet Autoantibody Accreditation Program of 98.9% (42). A Norwegian study of over 4000 individuals found anti-GAD has a prevalence of 1.7% in the non-diabetic Norwegian adult population (43) where it was associated with thyroid autoimmunity. Anti-GAD titre was higher in individuals with prediabetes than those with normal metabolic parameters. Our study used the highly sensitive agglutination PCR assay to detect subclinical levels of anti-GAD which were significantly higher than controls and were associated with progression to CIADM. It is plausible that patients with CIADM have a subclinical degree of anti-islet autoimmunity as evidenced by low titres of anti-GAD and anti-IAA reflecting subclinical islet autoimmunity that places them at risk once exposed to ICIs.

Our population of patients with CIADM had a 66% rate of T1D risk HLA-haplotypes among those who were tested. This is not substantially different to the background population rates of HLA risk-alleles and is substantially less than the 90-95% rate of high-risk alleles in people with T1D. It is worth noting that there is variability between frequency and composition of risk alleles in different series worldwide. This may relate, at least in part, to differences in HLA-types and T1D risk alleles between people of different ethnicity.

The use of pancreatic volumetry as a biomarker for prevalent type 1 diabetes and CIADM is established. Previous studies (20, 44, 45) in individuals at high risk of T1D have shown reduced pancreatic volume with progression to diabetes. Several studies have corroborated that CIADM is associated with a decline in pancreatic volume, however baseline, pre-ICI pancreatic volumes have not previously been reported in cases compared to controls (6, 46–48).

The strengths of this paper lie in the inclusion of longitudinal case control matched samples obtained and use of a diverse range of biomarker methodology. The biomarkers we have used in our final prediction model are all non-invasive, scalable, and easily accessible clinically through peripheral blood collection and CT scans that are already being conducted as part of routine care. Automated pancreatic volumetry methodology has previously been validated (49).

The limitations of this study include relatively low sample size, due to the relatively low incidence of CIADM at 0.4-1.9% of PD1/ PD-L1 ICI treated patients (5–9). Even so, this is the largest series of CIADM patients with longitudinal sample analyses. The lack of significant differentially expressed genes by RNA-sequencing of CD8+ cells was surprising. However, most of the flow cytometry identified differences were in CD4+ cells. After the CD8+ results were analysed, the CD4+ cells were no longer available to sequence, which is a limitation of this study. Inclusion of a Type 1 diabetes Genetic Risk Score (17) may further improve the ability to predict CIADM but this test is not routinely available.

A future validation study would allow testing of the robustness of the predictive variables identified in this study. Expanding the patient population to include other primary tumor types would be of interest. The sensitive auto-antibody detection assay used in this report (agglutination PCR assay (50)) found that 64% of CIADM patients have assay-detectable anti-GAD levels vs 14% of controls ($p<0.001$) and 54% have anti-insulin antibodies vs 21% of controls ($p=0.038$). Use of this assay, or another similarly sensitive assay and combining those results with CT or MRI examination of pancreatic volume is the most easily testable hypothesis. Although the circulating immune cell phenotypes added substantially to the predictive value, they will not be available in all centers.

The detection of subclinical anti-GAD titres and lower baseline pancreatic volume in our CIADM cohort suggests that CIADM patients have prior anti-islet immune responses that are poised under permissive conditions (i.e. immune checkpoint inhibition) to cause disease. That these patients have not developed T1D prior to the introduction of an anti-PD1 or anti-PDL1 inhibitor indicates that this immune pathway plays an important role in suppressing islet autoimmunity. The findings of higher Th17 helper cells, CD4+ central memory cells and lower CD4+ naïve cells at baseline with more activation on ICI introduction gives the impression of a more experienced and autoreactive immune system in CIADM patients compared to controls without immune-related adverse effects. Combined, these findings suggest that CIADM patients have a distinct immune profile that can be detected prior to ICI use.

The ability to predict IRAE has unique potential when the clinical indication for ICI is not definitively superior to alternatives. For example, in stage III melanoma, ICIs are currently considered alongside targeted therapy such as dabrafenib plus trametinib as effective adjuvant therapy and specific contraindications to ICIs such as autoimmune disease, immunosuppressive treatment guide choice of therapy (51). In this scenario, the ability to identify individuals at high risk of severe IRAE could further guide therapeutic choices in this area and reduce IRAE related morbidity. In people who have high likelihood of therapeutic benefit from ICIs but also a high risk of CIADM, knowledge of this risk would facilitate closer monitoring and thereby potentially prevent late diabetes diagnosis when ketoacidosis is present.

Conclusions

Immune-related adverse events are common in those treated with immune checkpoint inhibitors and vary in severity from mild to fatal. Prediction of immune-related adverse effects prior to therapy has the potential to inform clinical decisions, allow for earlier detection and open a potential window for prevention. Combining biomarkers from the fields of type 1 diabetes and immune-related adverse effects research, we have identified biomarkers that have potential to predict checkpoint inhibitor related autoimmune diabetes from baseline and on treatment characteristics. Prospective validation of these biomarkers is a crucial next step but a challenging prospect due to the relative low incidence of CIADM.

Methods

Sex as a biological variable

Of the patients studied, 11 people with CIADM were male and 3 female, and 22 ICI treated controls were male and 6 female. Melanoma has higher incidence in males.

Sample selection

Fourteen patients with CIADM and 28 ICI treated controls that had longitudinal biospecimens were identified from the prospectively collected Melanoma Institute of Australia medical record database (MRD2) and biospecimen bank.

The diagnosis of CIADM was based on new onset diabetes (HbA1c $\geq$6.5% and/or blood glucose $\geq$11mmol/L) in the setting of ICI therapy, with evidence of insulin deficiency (either presence of diabetic ketoacidosis or low C-peptide $\leq$0.4nmol/L with elevated glucose). No patients had previous diabetes.

Two controls were selected for each CIADM patient, matched as closely as possibly for age (±5 years), sex, type of immune checkpoint inhibitor therapy (single agent anti-PD1 versus combined anti-CTLA4 plus anti-PD1), time on therapy, treatment response and concurrent other IRAEs. If CIADM patients had no other IRAE, they were matched to controls without IRAE. If CIADM patients had other IRAE, they were matched to controls with those same IRAEs or if no such controls could be found, then a control with no IRAE.

Control patients had prospectively collected pre-ICI and on-ICI PBMC (~3 months after treatment initiation) and serum samples analysed. CIADM patients similarly had pre-ICI and on-treatment bloods collected at approximately 3 months. CIADM patients additionally had

samples taken approximately 3 months after CIADM diagnosis. A subgroup of control patients did not develop any immune-related adverse effects and they were also separately compared to assess the effect of general ICI related immune changes on various parameters. A summary of the methods is depicted in Figure 1.

Autoantibody analysis

Type 1 diabetes autoantibodies (anti-GAD, anti-IA2, anti-ZnT8 and anti-IA2) in serum samples were determined using agglutination PCR assay which has been previously described (50). Clinical thresholds for each autoantibody are set at the 98th percentile of results from 60-84 negative serum samples included in the 2023 International Islet Autoantibody Standardization Program (52).

Cytokine expression

Serum cytokine expression was measured using the Biolegend LEGENDplex^{TM} Human Essential Immune Response Multiplex Assay (Catalogue. No. 740930). This measures interleukins IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL17A, interferon (IFN)γ, tumor necrosis factor α (TNFα), CCL2, CXCL8 (IL-8), CXCL10 and free transforming growth factor β1 (TGFβ1). The assay was conducted in accordance with manufacturer's instructions with samples run in duplicate.

C-peptide assay

Serum C-peptide was measured using human C-peptide ELISA assay (CrystalChem, Catalog #80954) as per manufacturer's instructions.

Glucose levels

Serum glucose was measured directly from serum samples using Abbott Freestyle Libre glucometer and glucose test strips.

CT pancreatic volumetry

CT pancreatic volumetry was conducted by 1 investigator (L.W.) as previously published using Vitrea® software (Figure 1B). CT scans were obtained within 6 months of each blood collection timepoint. CT scans from CIADM cases were compared to the control cohort. Some of these pancreatic volumetry results have previously been published (53) and the expanded cohort is presented. CT scans were not available for one patient with CIADM and two controls. Most CIADM patients did not have protocol CT scans on-ICI before CIADM, so data is not available for that timepoint.

Flow cytometry

Cryopreserved PBMCs were thawed in media and washed in FACS buffer prior to staining. Samples were stained first with FVS700 Viability Dye (Cat no. 564997) in dark for 10 minutes, followed by human AB serum for 10 minutes. Antibodies were all purchased from BD Biosciences except CXCR5 PE-Cy7 which was from Biolegend. Cells were then stained with CCR6 BV480 (Cat no. 556130), CXCR3 PE-CF596 (Cat no. 562451), CCR7 BB700 (Cat no. 566438), and CXCR5 PE-Cy7 (Cat no. 356923) at 37°C for 15 minutes. Surface staining was then performed with CD45RA APC-H7 (Cat no. 560674), CD8 BUV496 (Cat no. 612943), CD127 BV786 (Cat no. 563324), CD3 BUV661 (Cat no. 612965), CD25 BB515 (Cat no. 564467), CD56 BUV737 (Cat no. 612767), CD16 BUV563 (Cat no. 741449), CD4 BUV805 (Cat no. 612887), CD38 BV421 (Cat no. 562445), HLA-DR BUV395 (Cat no. 565972) at 4°C for 30

minutes. Cells were analysed using the BD Symphony Analyser with gating strategy as shown in Supplementary Figure 1. A minimum of 30,000 cells were analysed per sample.

Cell sorting

Cryopreserved PBMCs were thawed and washed in FACS buffer prior to staining with FC block, CD45+, CD3+ CD8+, CD4+ and DAPI. CD8+ cells were identified via gating for CD45+ CD3+ CD8+ CD4- and DAPI negative subsets via the BD Influx cell sorter. 1000 CD8+ cells per samples were sorted per well into a 96 well plate and frozen down as per manufacturer's instructions.

RNA extraction and sequencing

Total RNA was extracted using Ultra Low Input Takarabio® kit. RNA was extracted and sequenced using a NovaSeq X with approximately 10 million 150bp paired end reads. RNA-Sequencing analysis was performed using R, using edgeR for differential gene analysis and STAR, RSEM, Tximport and DESeq2 with a Gencode 45 (latest) annotation for isoform analysis. Flow cytometry data were analysed using FlowJo®.

Statistics

Statistical analysis was performed using SPSS version 21, or GraphPad Prism version 10. Most serum, cytokine and flow cytometry data were not normally distributed, and were compared with Mann-Whitney where only 2 datasets were compared, or Kruskall-Wallis testing with Dunn's correction for multiple comparisons where >2 sets were examined. In the case of matching samples, e.g. pre-ICI, on-ICI and CIADM in the same people, Wilcoxon signed-rank test was used, again, corrected for multiple comparisons. Normally distributed

data were compared using one-way ANOVA with correction for multiple comparisons. Multiple-comparison adjusted p-values of <0.05 were considered statistically significant. When comparing paired data across time-courses, Wilcoxon signed-rank test was used with manual addition of the correction for the number of comparisons with Bonferroni.

IBM SPSS Statistics version 28 was used to analyse the variables demonstrating univariate association with diabetes status. These were candidates for inclusion in multivariate binary logistic regression models. Backward stepwise variable selection was used to identify the independent predictors of diabetes status in the best fitting multivariate logistic regression model. The area under the receiver operating curve (ROC) was utilized to evaluate the performance of the fitted model from the best multivariate logistic regression model to correctly classifying a patient's diabetes status.

P values of <0.05 after any corrections for multiple comparisons were taken as significant. Illustrations were made using Adobe Illustrator or GraphPad Prism.

Study approval

All patients gave written informed consent. The study was approved by Royal Prince Alfred Hospital Research Ethics Committee, Sydney on Protocol No. X10-0305 and HREC/10/RPAH.

Data availability

Data other than original sequencing files will be able to be accessed from FigShare using doi 10.6084/m9.figshare.29453093 from the date of online publication. Normalized RNA sequencing data is available at the same FigShare location. Please email the corresponding author for access to the original sequencing files.

Author contributions

LW, JMW, CL, NF, MC, DAB, RC, GVL, RAS, SCS, VHMT, AMM and JEG contributed to study design. Patients were recruited to the database and biobank by MC, GVL, RAS, and AMM. LW, JMW, and NF conducted experiments and acquired data. Data analysis was performed by LW, JMW, CL, NF, NN, and JEG. Figures were prepared by LW, NN, and JEG. All authors contributed to writing and revising the manuscript.

Acknowledgements

We would like to thank Westmead Institute for Medical Research Flow Cytometry department and Genomics department for assistance and advice. We would like to thank Westmead Hospital Department of Radiology for allowing use of Vitrea® software. We would like to thank Sabrina Naz from Research and Education Network (REN), Westmead Hospital, Sydney, for assistance with statistics.

JEG is supported by NHMRC Investigator grant 2033228 and was supported by NHMRC Program grant 1149976. AMM is supported by NHMRC Investigator grant 2009476. GVL is supported by NHMRC Investigator grant 2007839.

Disclosures

JW received consulting fees or honoraria from Sanofi, SAB Biosciences and Lilly.

DAB receives royalties from Roche for GDF15 assays, which have been used as a biomarker of diabetes development.

GVL is consultant advisor for Agenus, Amgen, Array Biopharma, AstraZeneca, Bayer, BioNTech, Boehringer Ingelheim, Bristol Myers Squibb, Evaxion, Hexal AG (Sandoz Company), Highlight Therapeutics S.L., IOBiotech, Immunocore, Innovent Biologics USA, Merck Sharpe & Dohme, Novartis, PHMR Ltd, Pierre Fabre, Regeneron.

RAS receives royalties from book sales AFIP Atlases of Tumour and Non-Tumor Pathology, Fifth Series, Fascicle 19, Melanocytic Tumors of the Skin, Pathology of Melanocytic Tumors and consulting fees from SkylineDx BV, IO Biotech ApS, MetaOptima Technology Inc., F. Hoffmann-La Roche Ltd, Evaxion, Provectus Biopharmaceuticals Australia, Qbiotics, Novartis, Merck Sharp & Dohme, NeraCare, AMGEN Inc., Bristol-Myers Squibb, Myriad Genetics, and GlaxoSmithKline.

MC has served on advisory boards or as a consultant for Amgen, Bristol-Myers Squibb, Eisai, Ideaya, Merck Sharpe & Dohme, Nektar, Novartis, Oncosec, Pierre-Fabre, Qbiotics, Regeneron, Roche, Merck, and Sanofi, and received honoraria from Bristol-Myers Squibb, Merck Sharpe & Dohme, and Novartis.

RCB has served as a consultant or speaker and received honoraria from Amgen, Eisai, Ipsen, and Specialized Therapeutics.

AMM has served on advisory boards for BMS, MSD, Novartis, Roche, Pierre-Fabre, QBiotics.

PR has received honoraria for lectures and Advisory Boards from MSD, BMS, Novartis, Pierre Fabre, Merck, Genesis Pharma, and Medison Pharma.

References

1. Paul J, et al. Overlapping and non-overlapping indications for checkpoint inhibitors in the US. 2024;11057. https://doi.org/10.1200/JCO.2024.42.16_suppl.11057

2. Menzies AM, et al. Pathological response and survival with neoadjuvant therapy in melanoma: A pooled analysis from the International Neoadjuvant Melanoma Consortium (INMC). *J Clin Oncol*. 2019;37(15_suppl):9503–9503.

3. Patel SP, et al. Neoadjuvant–Adjuvant or Adjuvant-Only Pembrolizumab in Advanced Melanoma. *N Engl J Med*. 2023;388(9):813–823.

4. Wu L, et al. Risk Factors and Characteristics of Checkpoint Inhibitor-Associated Autoimmune Diabetes Mellitus (CIADM): A Systematic Review and Delineation From Type 1 Diabetes. *Diabetes Care*. 2023;46(6):1292–1299.

5. Tsang VHM, et al. Checkpoint Inhibitor-Associated Autoimmune Diabetes Is Distinct from Type 1 Diabetes. *J Clin Endocrinol Metab*. 2019;104(11):5499–5506.

6. Byun DJ, et al. Immune checkpoint inhibitor– associated diabetes: A single-institution experience. *Diabetes Care*. 2020;43(12):3106–3109.

7. Wright JJ, et al. Increased reporting of immune checkpoint inhibitor-associated diabetes. *Diabetes Care*. 2018;41(12):e150–e151.

8. De Filette JMK, et al. Immune checkpoint inhibitors and type 1 diabetes mellitus: A case report and systematic review. *Eur J Endocrinol*. 2019;181(3):363–374.

9. Barroso-Sousa R, et al. Incidence of endocrine dysfunction following the use of different immune checkpoint inhibitor regimens a systematic review and meta-analysis. *JAMA Oncol*. 2018;4(2):173–182.

10. Chaput N, et al. Baseline gut microbiota predicts clinical response and colitis in metastatic melanoma patients treated with ipilimumab. *Ann Oncol*. 2017;28(6):1368–1379.

11. Nuñez NG, et al. Immune signatures predict development of autoimmune toxicity in patients with cancer treated with immune checkpoint inhibitors. *Med*. 2023;4(2):113-129.e7.

12. Subudhi SK, et al. Clonal expansion of CD8 T cells in the systemic circulation precedes development of ipilimumab-induced toxicities. *Proc Natl Acad Sci U S A*. 2016;113(42):11919–11924.

13. Oh DY, et al. Immune toxicities elicted by CTLA-4 blockade in cancer patients are associated with early diversification of the T-cell repertoire. *Cancer Res*. 2017;77(6):1322–1330.

14. Khan S, et al. Immune dysregulation in cancer patients developing immune-related adverse events. *Br J Cancer*. 2019;120(1):63–68.

15. Tarhini AA, et al. Baseline circulating IL-17 predicts toxicity while TGF-β1 and IL-10 are prognostic of relapse in ipilimumab neoadjuvant therapy of melanoma. *J Immunother Cancer*. 2015;3(1):15–20.

16. Fujisawa Y, et al. Fluctuations in routine blood count might signal severe immune-related adverse events in melanoma patients treated with nivolumab. *J Dermatol Sci*. 2017;88(2):225–231.

17. Ruiz-Esteves KN, et al. Identification of Immune Checkpoint Inhibitor–Induced Diabetes. *JAMA Oncol*. 2024;02114(10):1409–1416.

18. Ziegler AG, et al. Seroconversion to Multiple Islet Autoantibodies and Risk of Progression to Diabetes in Children. *JAMA*. 2013;309(23):2473.

19. Redondo MJ, et al. A type 1 diabetes genetic risk score predicts progression of islet autoimmunity and development of type 1 diabetes in individuals at risk. *Diabetes Care*. 2018;41(9):1887–1894.

20. Virostko J, et al. Longitudinal Assessment of Pancreas Volume by MRI Predicts Progression to Stage 3 Type 1 Diabetes. *Diabetes Care*. 2024;47(3):393–400. https://doi.org/10.2337/dc23-1681

21. Shapiro MR, et al. Human immune phenotyping reveals accelerated aging in type 1 diabetes. *JCI Insight*. 2023;8(17). https://doi.org/10.1172/jci.insight.170767.

22. Kenefeck R, et al. Follicular helper T cell signature in type 1 diabetes. *J Clin Invest*. 2015;125(1):292–303.

23. Ferreira RC, et al. IL-21 production by CD4+ effector T cells and frequency of circulating follicular helper T cells are increased in type 1 diabetes patients. *Diabetologia*. 2015;58(4):781–790.

24. Shek D, et al. Non-coding RNA and immune-checkpoint inhibitors: Friends or foes? *Immunotherapy*. 2020;12(7):513–529.

25. Long SA, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4+CD25+ regulatory T-cells of type 1 diabetic subjects. *Diabetes*. 2010;59(2):407–415.

26. Mallone R, et al. CD8+ T-cell responses identify β-cell autoimmunity in human type 1 diabetes. *Diabetes*. 2007;56(3):613–621.

27. Herold KC, et al. Validity and reproducibility of measurement of islet autoreactivity by T-cell assays in subjects with early type 1 diabetes. *Diabetes*. 2009;58(11):2588–2595.

28. Hughes J, et al. Precipitation of autoimmune diabetes with anti-PD-1 immunotherapy. *Diabetes Care*. 2015;38(4):e55–e57.

29. Kovacsovics-Bankowski M, et al. Lower frequencies of circulating suppressive regulatory T cells and higher frequencies of CD4 + naïve T cells at baseline are associated with severe

immune-related adverse events in immune checkpoint inhibitor-treated melanoma. *J Immunother Cancer*. 2024;12(1):1–13.

30. Lozano AX, et al. T cell characteristics associated with toxicity to immune checkpoint blockade in patients with melanoma. *Nat Med*. 2022;28(2):353–362.

31. Bukhari S, et al. Single-cell RNA sequencing reveals distinct T cell populations in immune-related adverse events of checkpoint inhibitors. *Cell Reports Med*. 2023;4(1):100868.

32. Kim KH, et al. Immune-related adverse events are clustered into distinct subtypes by T-cell profiling before and early after anti-PD-1 treatment. *Oncoimmunology*. 2020;9(1). https://doi.org/10.1080/2162402X.2020.1722023.

33. Honkanen J, et al. IL-17 Immunity in Human Type 1 Diabetes. *J Immunol*. 2010;185(3):1959–1967.

34. Kuriya G, et al. Double deficiency in IL-17 and IFN-γ signalling significantly suppresses the development of diabetes in the NOD mouse. *Diabetologia*. 2013;56(8):1773–1780.

35. Reinert-Hartwall L, et al. Th1/Th17 Plasticity Is a Marker of Advanced β Cell Autoimmunity and Impaired Glucose Tolerance in Humans. *J Immunol*. 2015;194(1):68–75.

36. Tatovic D, et al. Ustekinumab for type 1 diabetes in adolescents: a multicenter, double-blind, randomized phase 2 trial. *Nat Med*. 2024;30(September). https://doi.org/10.1038/s41591-024-03115-2.

37. Kent SC, et al. Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope. *Nature*. 2005;435(7039):224–228.

38. Gauci ML, et al. Autoimmune diabetes induced by PD-1 inhibitor—retrospective analysis and pathogenesis: a case report and literature review. *Cancer Immunol Immunother*. 2017;66(11):1399–1410.

39. Godwin JL, et al. Nivolumab-induced autoimmune diabetes mellitus presenting as

diabetic ketoacidosis in a patient with metastatic lung cancer. *J Immunother Cancer*. 2017;5(1):1–7.

40. Lowe JR, et al. Genetic risk analysis of a patient with fulminant autoimmune type 1 diabetes mellitus secondary to combination ipilimumab and nivolumab immunotherapy. *J Immunother Cancer*. 2016;4(1):1–8.

41. Stamatouli AM, et al. Collateral damage: Insulin-dependent diabetes induced with checkpoint inhibitors. *Diabetes*. 2018;67(8):1471–1480.

42. Lampasona V, et al. Islet Autoantibody standardization program 2018 Workshop: Interlaboratory comparison of glutamic acid decarboxylase autoantibody assay performance. *Clin Chem*. 2019;65(9):1141–1152.

43. Sørgjerd EP, et al. Presence of anti-GAD in a non-diabetic population of adults; time dynamics and clinical influence: results from the HUNT study. *BMJ Open Diabetes Res Care*. 2015;3(1):e000076.

44. Williams AJK, et al. Pancreatic volume is reduced in adult patients with recently diagnosed type 1 diabetes. *J Clin Endocrinol Metab*. 2012;97(11):2109–2113.

45. Roger R, et al. Deep learning-based pancreas volume assessment in individuals with type 1 diabetes. *BMC Med Imaging*. 2022;22(1):1–5.

46. Marchand L, et al. Diabetes mellitus induced by PD-1 and PD-L1 inhibitors: description of pancreatic endocrine and exocrine phenotype. *Acta Diabetol*. 2019;56(4):441–448.

47. Wei HH, et al. Distinct changes to pancreatic volume rather than pancreatic autoantibody positivity: insights into immune checkpoint inhibitors induced diabetes mellitus. *Diabetol Metab Syndr*. 2024;16(1):1–10.

48. Perdigoto AL, et al. Immune cells and their inflammatory mediators modify β cells and cause checkpoint inhibitor–induced diabetes. *JCI Insight*. 2022;7(17).

https://doi.org/10.1172/jci.insight.156330.

49. Lim SH, et al. Automated pancreas segmentation and volumetry using deep neural network on computed tomography. *Sci Rep*. 2022;12(1):1–9.

50. Sing ABE, et al. Feasibility and Validity of In-Home Self-Collected Capillary Blood Spot Screening for Type 1 Diabetes Risk. *Diabetes Technol Ther*. 2024;26(2):87–94.

51. Funck-Brentano E, et al. Which adjuvant treatment for patients with BRAFV600-mutant cutaneous melanoma? *Ann Dermatol Venereol*. 2021;148(3):145–155.

52. Auchenbach P L V. IASP Autoantibody Workshop. *IDS Sci Meet Paris*. 2023.

53. Wu L, et al. Checkpoint Inhibitor-Associated Autoimmune Diabetes Mellitus Is Characterized by C-peptide Loss and Pancreatic Atrophy. *J Clin Endocrinol Metab*. 2024;109(5):1301–1307.

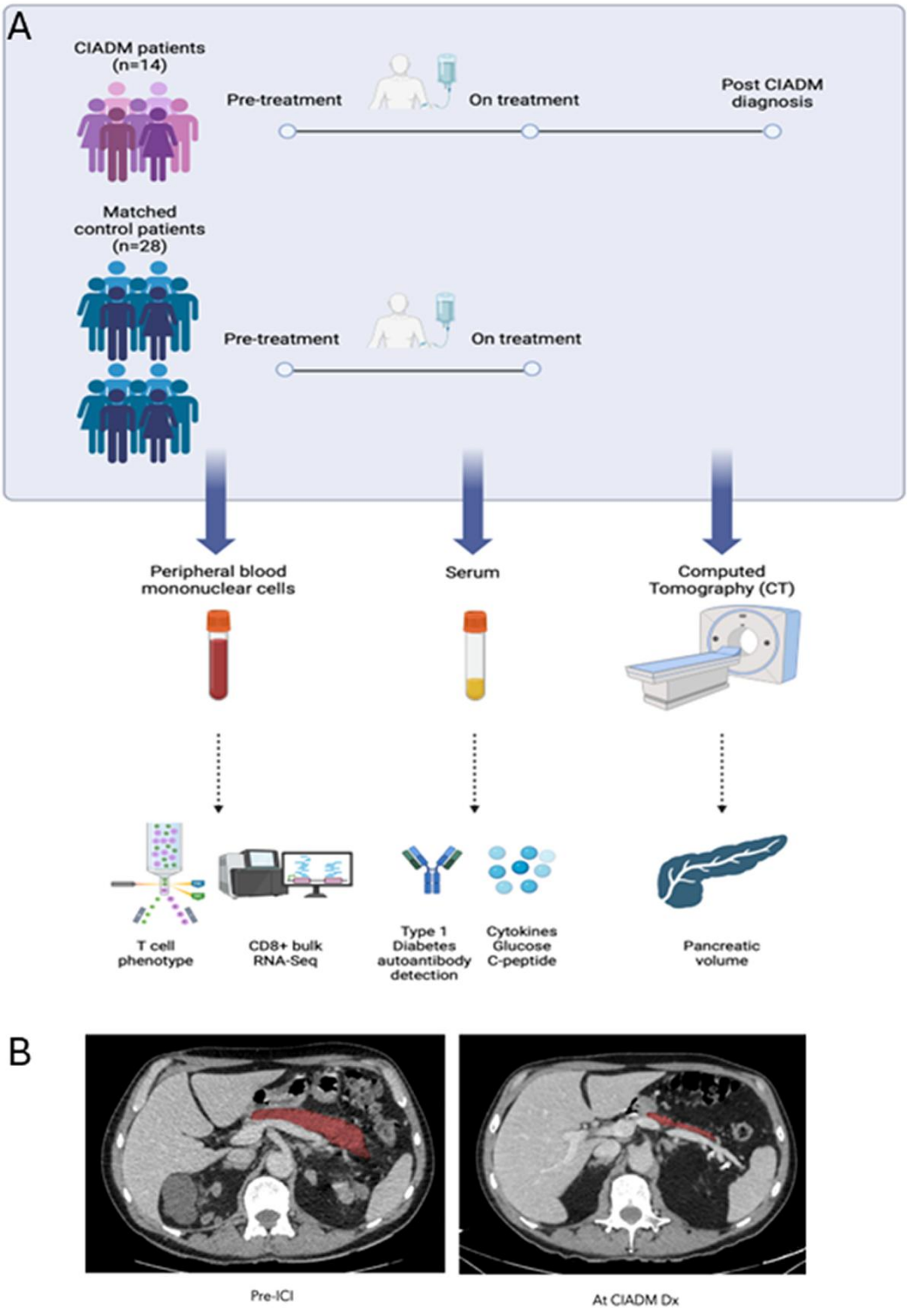
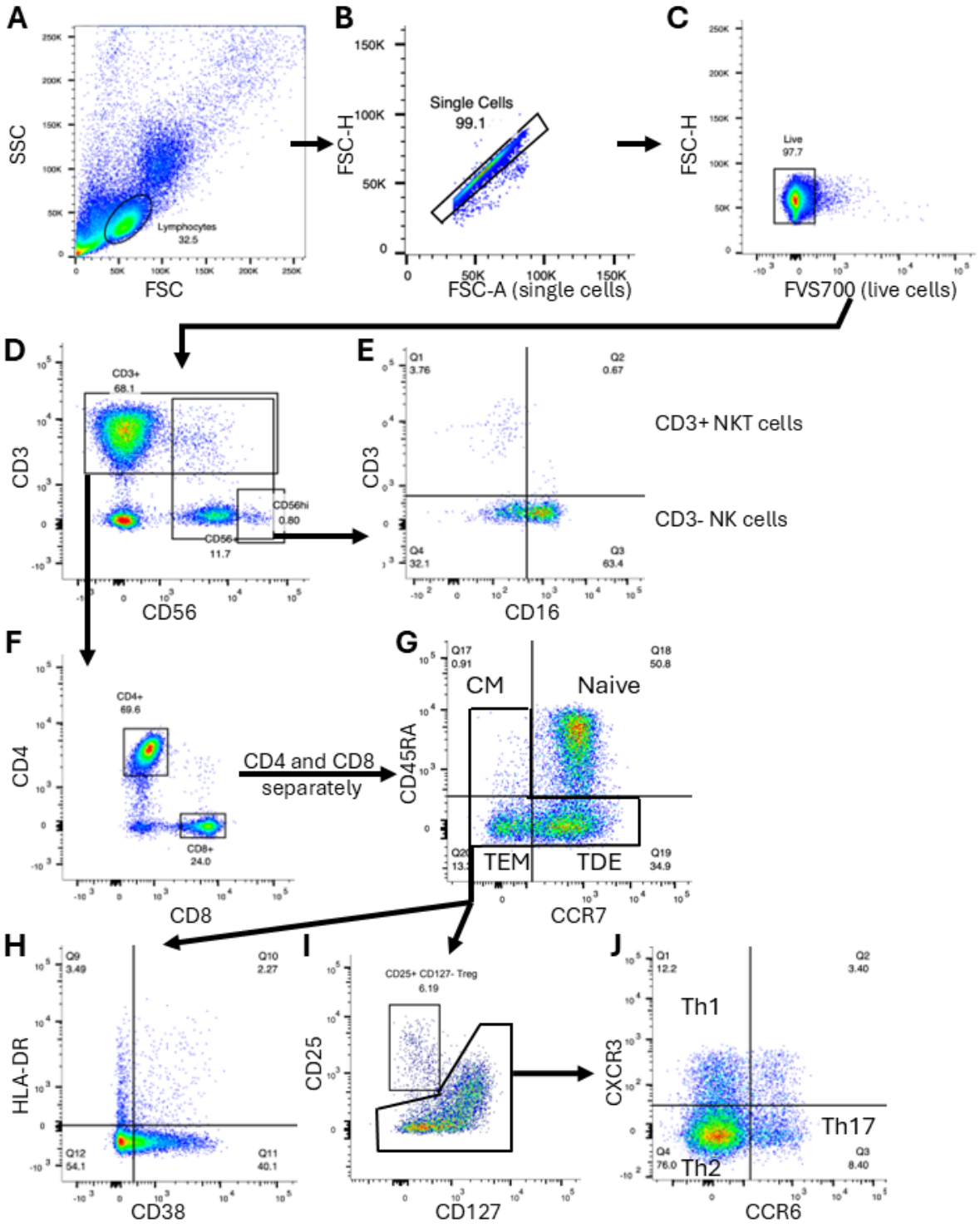


Figure 1.A. Summary of methodology. **B.** Representative CT scans of a patient with CIADM prior to ICI therapy and at time of CIADM diagnosis (red = pancreatic area).



Supplementary Figure 1. Gating strategy for T and NKT phenotyping in PBMC. Cells were gated on **A)** Lymphocytes, **B)** single cells, **C)** Live cells, **D)** CD56+ cells were distinguished from CD3+ cells. **E)** CD56+ cells were gated on CD3 and CD16+ status to identify CD3+ NKT and CD3- NK cells. **F)** CD3+ cells were gated by CD4+ or CD8+. **G)** CD4+ and CD8+ T-cells were separately gated on CD45RA versus CCR7 to identify central memory (CM), naïve T cells, T effector memory (TEM) and terminally differentiated effector (TDE) cells. **H)** Activation markers HLA-DR and CD38 were used to gate % activated T cells in the CD4+ and CD8+ subsets. **I)** CD4+Cd25+ CD127- T regulatory cells were gated. **J)** CD4+ cells were gated with CXCR3 and CCR6 to identify Th1, Th2 and Th17 status.

Table 1. Baseline characteristics.

	CIADM (n=14)	Control (n=28)
Mean age (years; +/- SD)	71.2 (12.3)	67.2 (12.1)
Sex (M/F)	11 (78%) / 3 (22%)	22 (78%) / 6 (22%)
Prior autoimmune disease	1	1
Type of ICI therapy		
Anti-PD1	7 (50%)	14 (50%)
Anti-PD1 plus anti-CTLA4	7 (50%)	14 (50%)
Metastatic disease	14 (100%)	28 (100%)
Prior exposure to other anti-cancer therapy (e.g. TKI, chemotherapy)	7 (50%)	14 (50%)
Response to ICI therapy		
Complete response	7 (50%)	14 (50%)
Partial response	4 (29%)	8 (29%)
Stable disease	0	0
Progressive disease	3 (21%)	6 (21%)
Immune-related adverse effects	14 (100%)	17 (60%)
Thyroiditis	8	6
Skin	4	4
Colitis	3	2
Pancreatitis	3	0
Hepatitis	2	2

Type 1 diabetes high-risk HLA DR/DQ haplotypes	6 of 9 tested (66.7%). N=3 DR3, N=1 DR4, N=1 DR3/4, N=1 DR13	N/A
Type 1 resistant HLA haplotypes	2 (22.2%, 1 patient with DR3 haplotype and 1 with no risk haplotypes)	N/A
Time to CIADM (weeks, median (IQR))	32 (7-65)	N/A

TKI = tyrosine kinase inhibitor. IQR = interquartile range.

Supplementary Table 1. Significant variables for pre-ICI comparisons. These variables were input into multiple logistic regression.

	CIADM	Control	Test, p-value
Baseline pancreatic volume (mls)	56±8	77±5	T-test p=0.023
Anti-GAD	2.9 (0-6.1)	0 (0-0.8)	M-W p=0.0021
Anti-IAA	2.0 (1.4-2.4)	1.0 (0-1.78)	M-W p=0.048
% CD4+ central memory	4.3 (2.4-11.8)	1.7 (0.7-3.7)	M-W p=0.01
% CD4+ naïve	38.5 (32.7-44.2)	50.8 (39.9-60)	M-W p=0.03
% Th17 cells	11 (8.5-13.2)	6.6 (5.6-7.9)	M-W p=0.001
% CD8+HLA-DR+CD38+	0.8 (0.6-2.0)	2 (1.3-3.1)	M-W p=0.014
% NK CD56hi	1.6 (0.8-2.6)	0.5 (0.3-1.1)	M-W p=0.0065

Data which is normally distributed is presented as mean±SEM and non-parametric data is shown with median and interquartile range (IQR). M-W = Mann Whitney

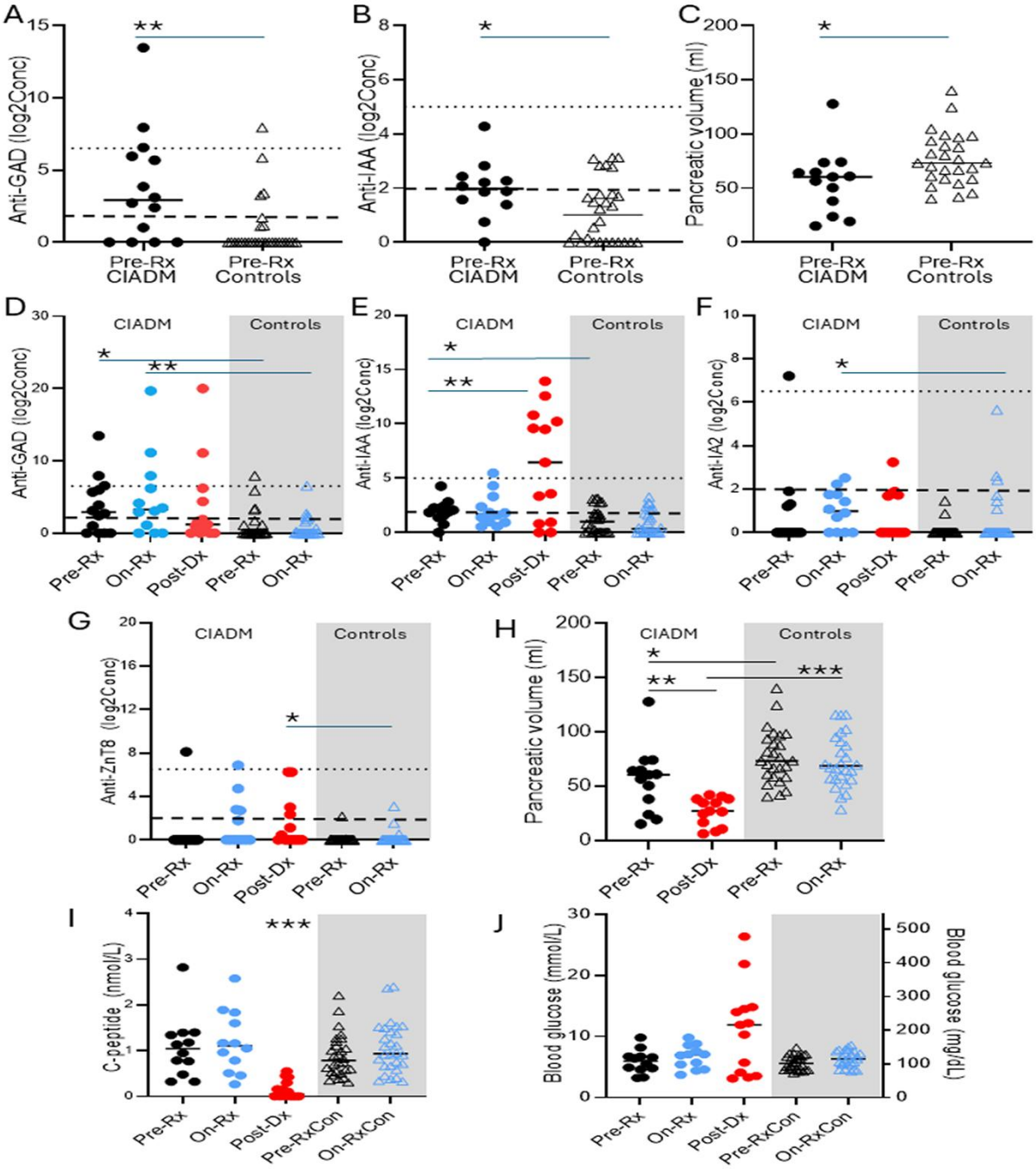


Figure 2. Antibody levels, pancreatic volume, C-peptide and glucose. **A)** Anti-GAD antibodies before ICI in control and CIADM. **B)** Anti-IAA pre-ICI. **C)** Pancreatic volume pre-ICI. **D)** Anti-GAD before and on-ICI. **E)** Anti-IAA before and on-ICI. **F)** Anti-IA2 before and on-ICI. **G)** Anti-ZnT8 before and on-ICI. **H)** Pancreatic volumes before and on-ICI. **I)** C-peptide before and on-ICI. **J)** Blood glucose before and on-ICI. Lines indicate median. Dotted lines at A, B, and D-G indicate thresholds for positive. *p<0.05, **p<0.01. ***p<0.0001 for indicated comparison. For **A-C**, Mann-Whitney tests. For **D-J** Kruskall-Wallis corrected for multiple comparison with Dunn's test.

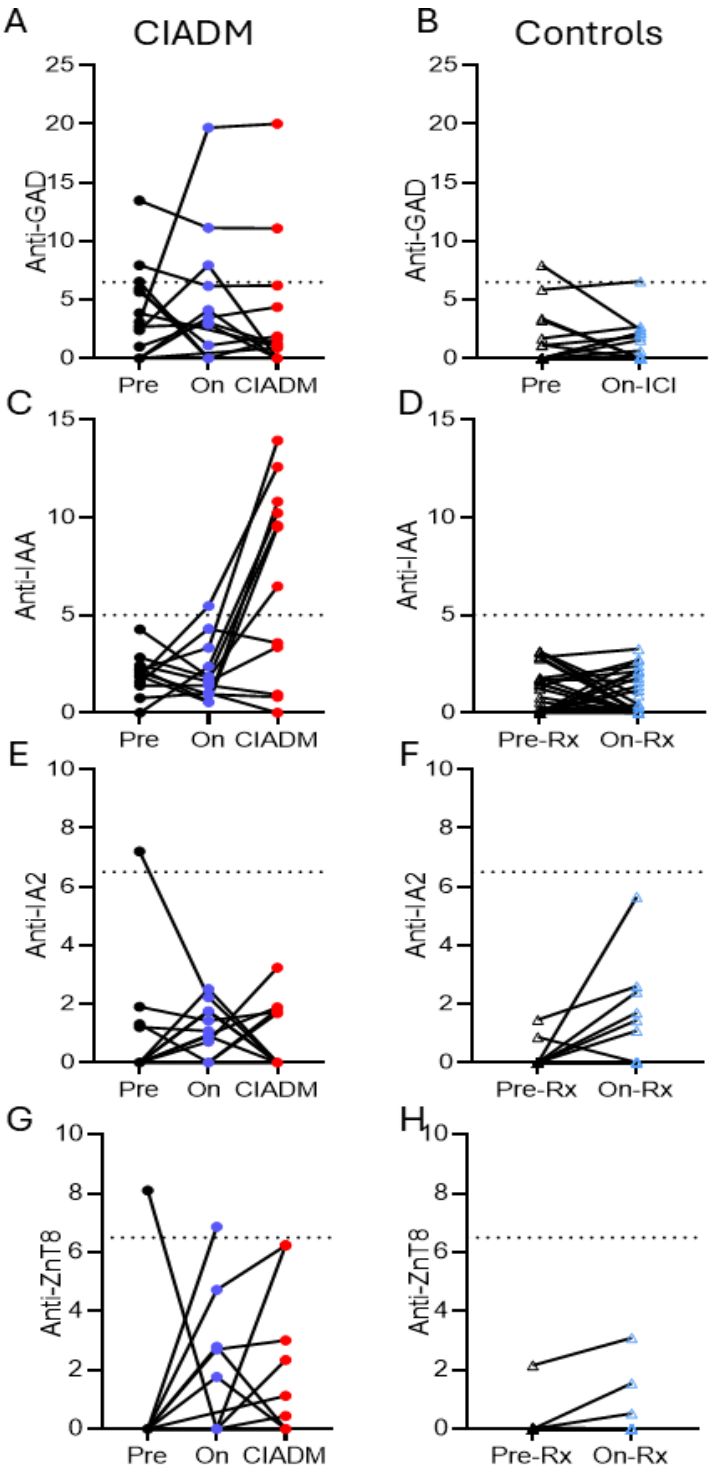


Figure 3. Changes in antibody titre with time. **A) and B)** Anti-GAD antibodies. **C) and D)** Anti-IAA antibodies. **E) and F)** Anti-IA2 antibodies. **G) and H)** Anti-ZnT8 antibodies. Note the majority of people who did not develop CIADM (Controls) were 0 to 0 titres for all antibodies except IA2, so their results align across the x-axis. We note that developing insulin auto-antibodies after commencing insulin therapy is common, as was observed in the CIADM group after starting insulin.

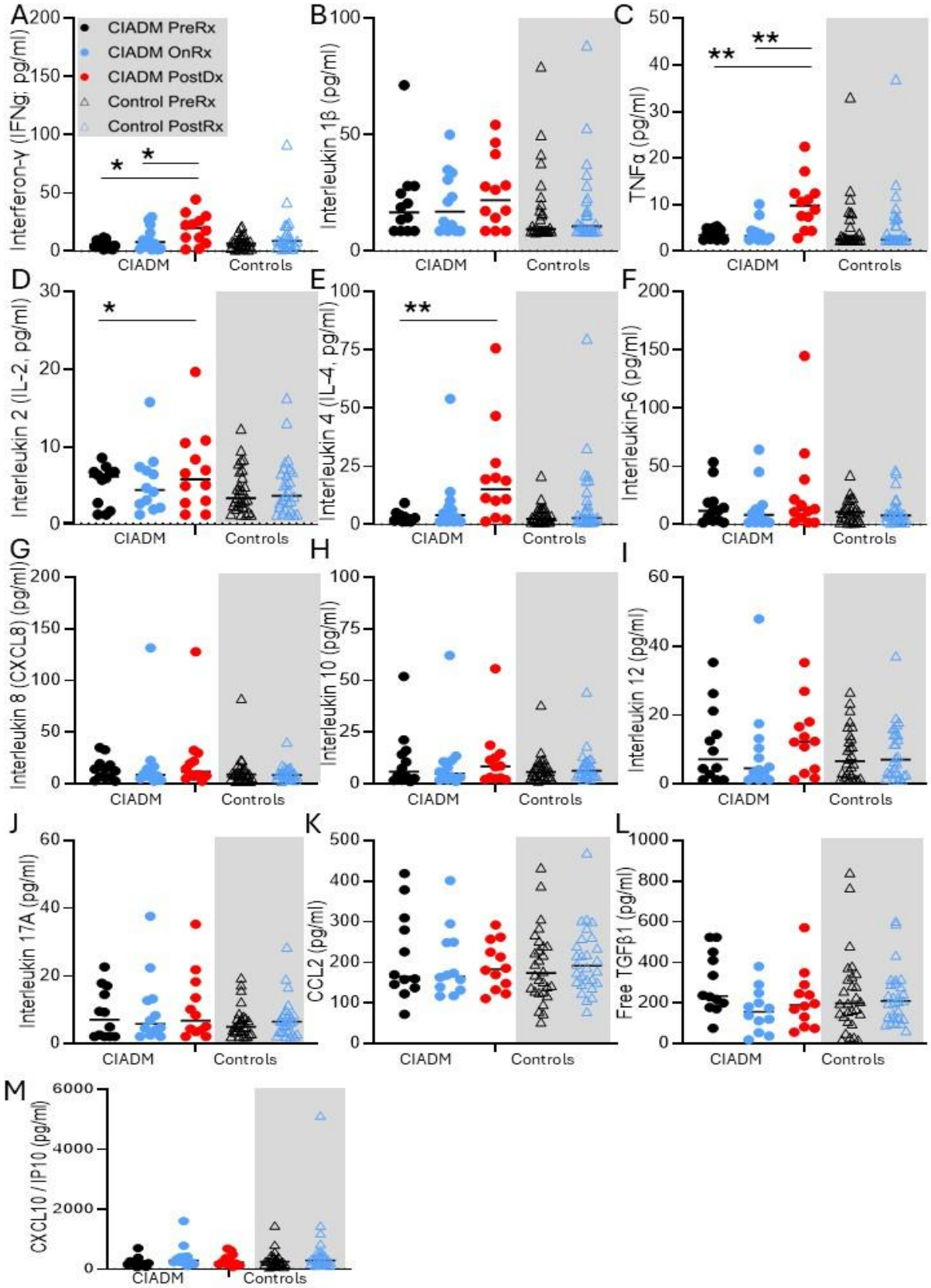


Figure 4. Circulating cytokines. **A)** IFNγ, **B)** IL1β, **C)** TNFα, **D)** IL2, **E)** IL4, **F)** IL6, **G)** IL8, **H)** IL10, **I)** IL12, **J)** IL17A, **K)** CCL2, **L)** Free TGFβ1, **M)** CXCL10. * p<0.05, **p<0.01 for the indicated comparison, Kruskall-Wallis with Dunn's correction for multiple comparisons. Lines indicate median.

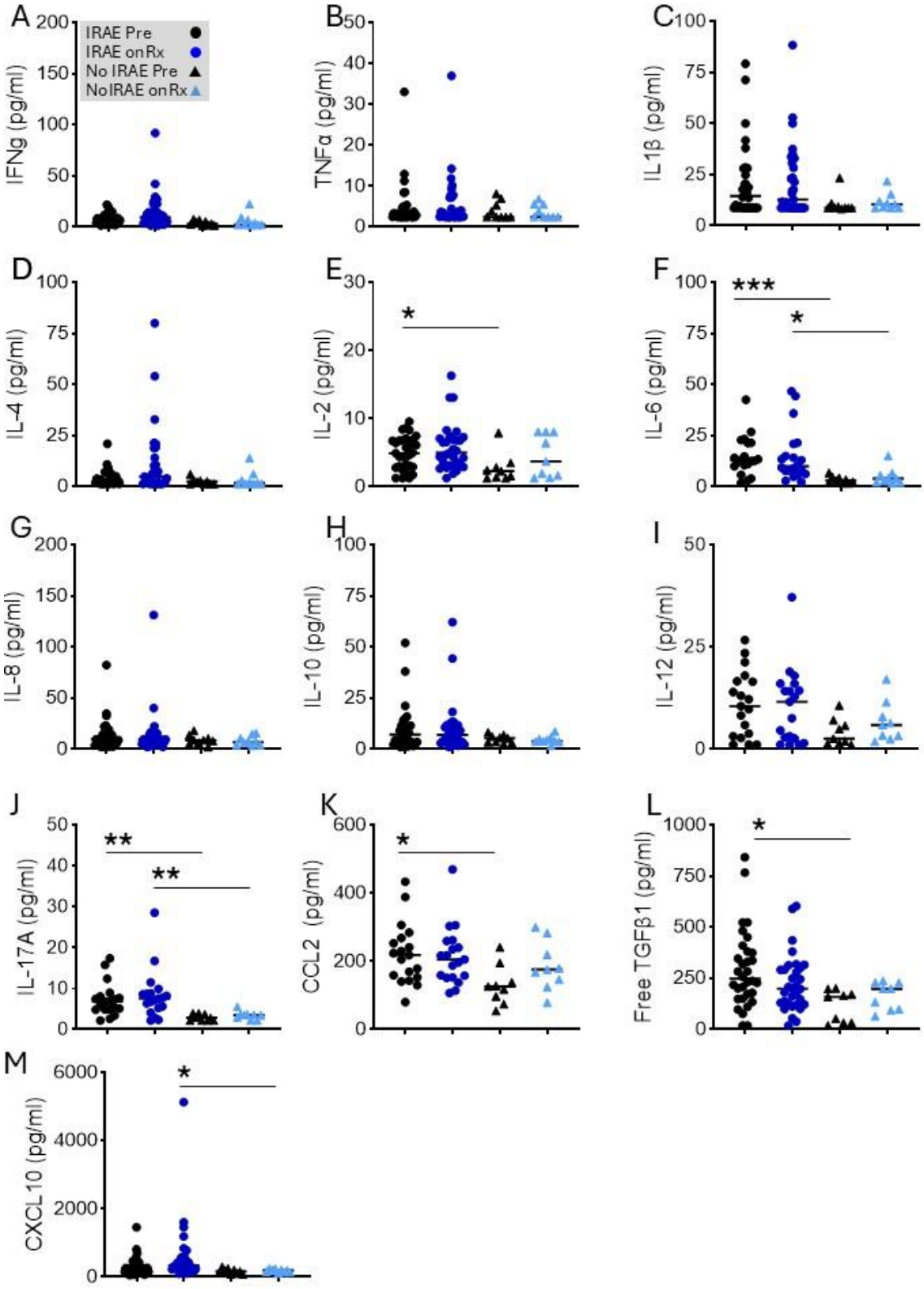


Figure 5. Cytokine levels in patients without immune related adverse effects (IRAE) pre and on-ICI and in people with IRAE pre and post-ICI. **A)** IFNγ, **B)** IL1β, **C)** TNFα, **D)** IL2, **E)** IL4, **F)** IL6, **G)** IL8, **H)** IL10, **I)** IL12, **J)** IL17A, **K)** CCL2, **L)** Free TGFβ1, **M)** CXCL10. *p<0.05 and ** p<0.01 for indicated comparison by Kruskal-Wallis testing with Dunn's correction for multiple comparisons.

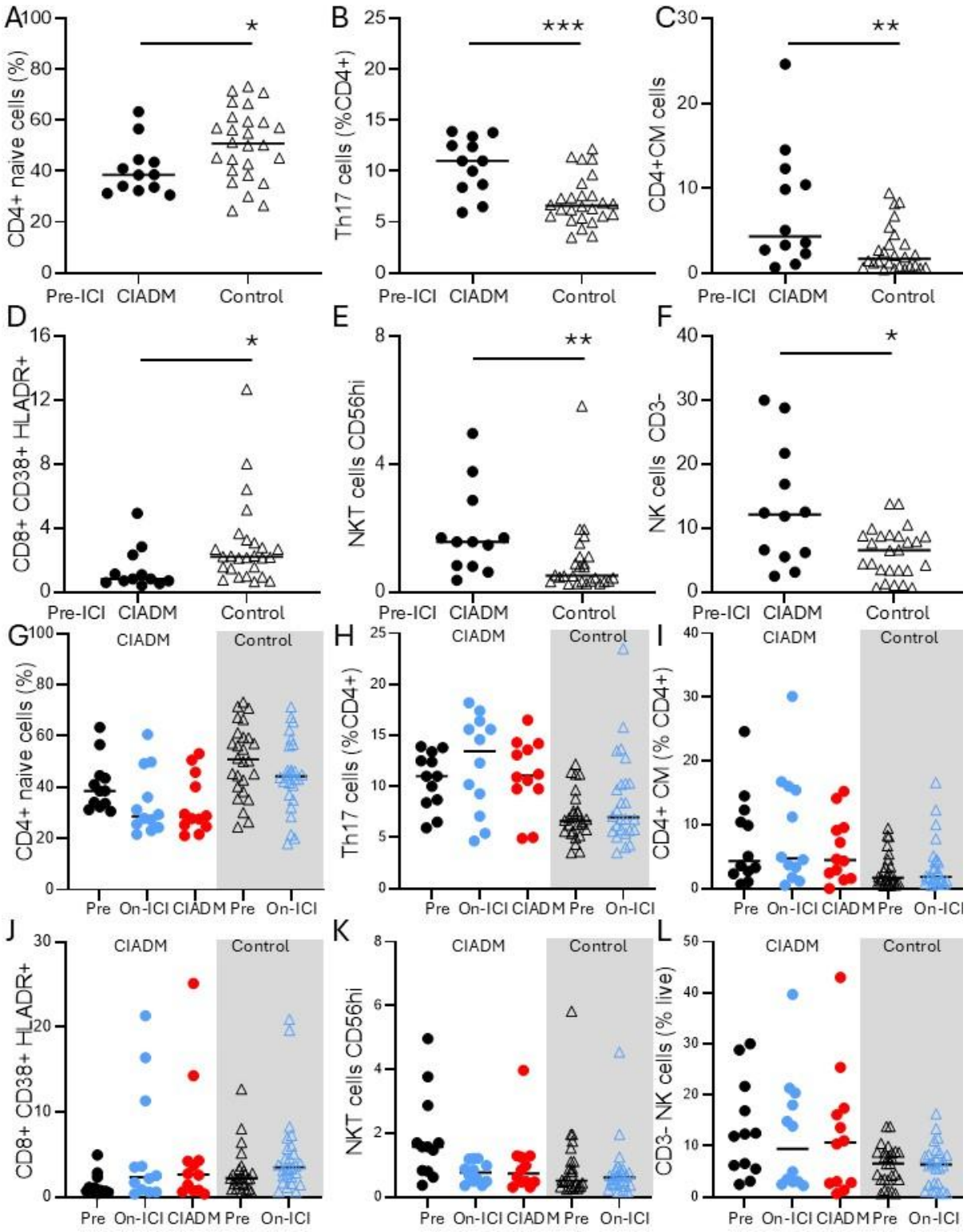


Figure 6. Flow cytometry immune cell subsets. **Figures A-F** depict pre-ICI differences between CIADM and control patients, **Figures G-L** depict data across all time points. * $p<0.05$, ** $p<0.01$ for the indicated comparison, Kruskall-Wallis with Dunn's correction for multiple comparisons. Lines indicate median.

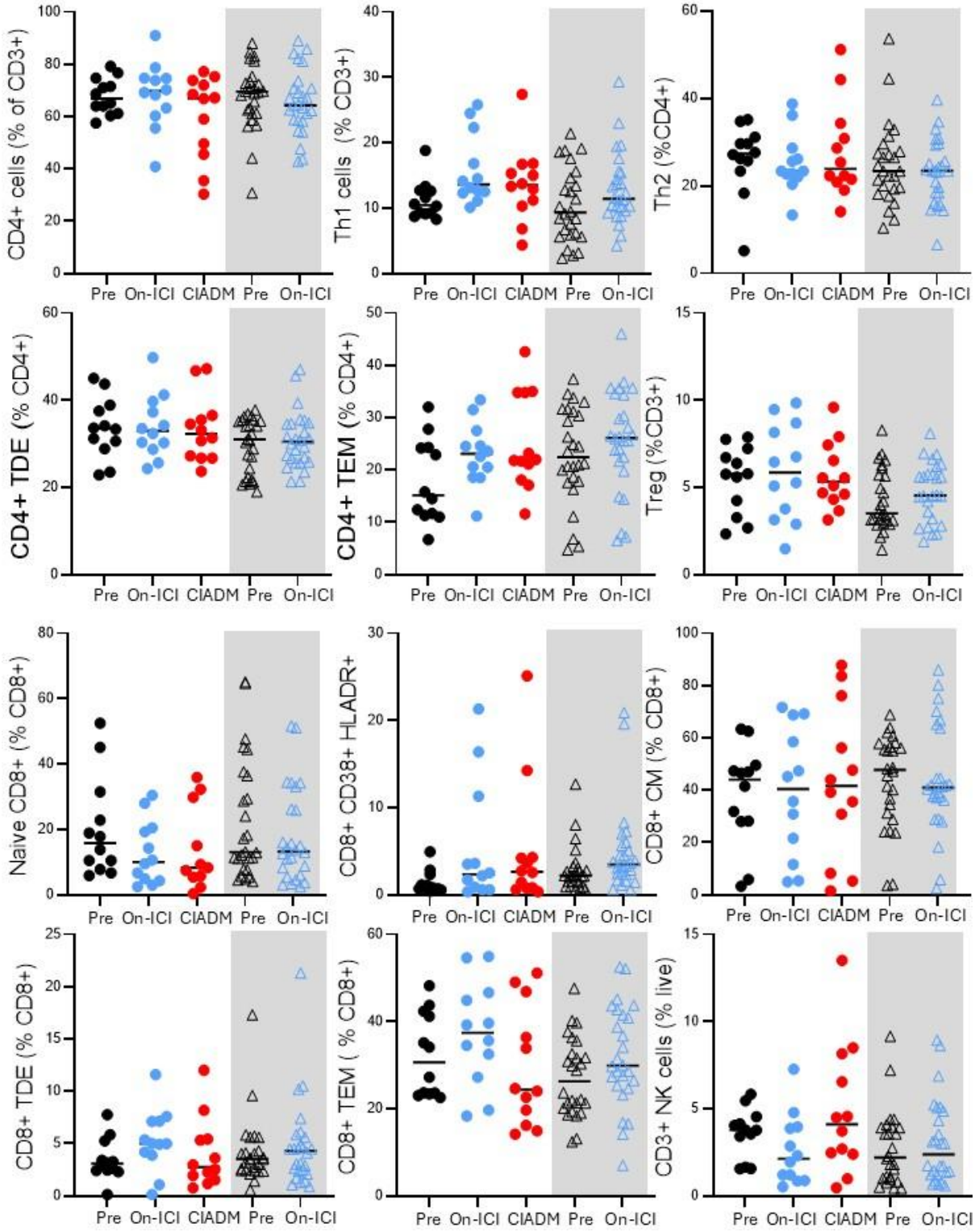


Figure 7. Additional flow cytometry results. **A)** All CD4+ cells, as a percentage of T-cells. **B)** Th1 T-cells. **C)** Th2 T-cells. **D)** CD4+ terminally differentiated effector cells (TDE). **E)** CD4+ T-effector memory (TEM). **F)** Regulatory T cells (Treg). **G)** CD*+ naïve cells. **H)** Innate-like bystander activated T-cells (CD8+ CD38+ HLADR+ cells). **I)** CD8+ central memory (CM) cells. **J)** CD8+ TDE cells. **K)** CD8+ TDE cells. **K)** CD3+ NK cells. The grey shaded areas show control ICI-treated patients. No differences were statistically significant after correction for multiple comparisons.

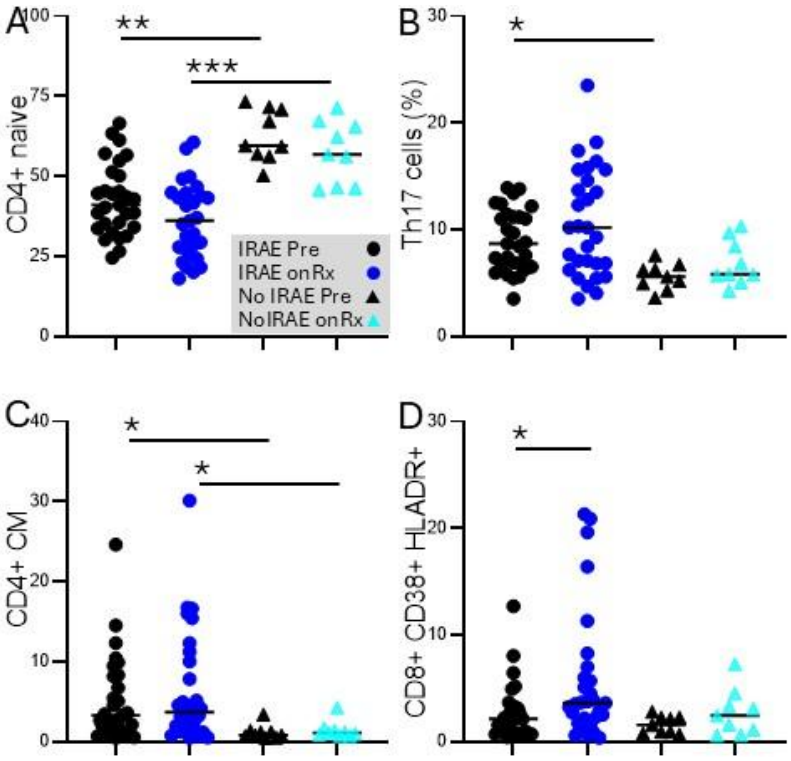


Figure 8. Immune cell subtypes in people with and without IRAE (immune related adverse effects). **A)** CD4+ naïve T cells. **B)** Th17 cells. **C)** CD4+ central memory cells. **D)** CD8+ CD38+ HLADR+ cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ for indicated comparison by Kruskal-Wallis testing with correction for multiple comparisons.

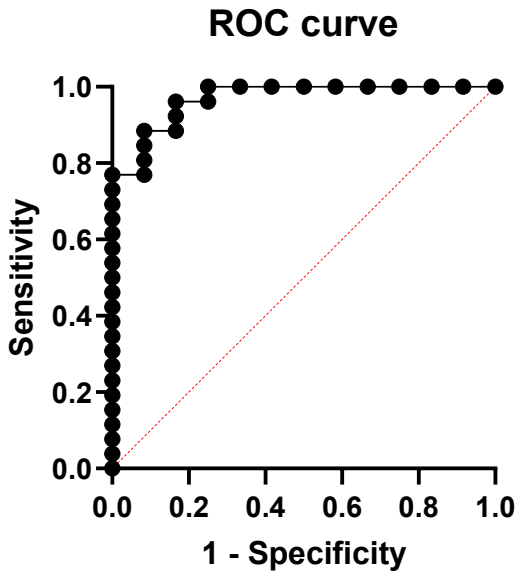


Figure 9. ROC curve for multiple logistic regression predicting CIADM diagnosis from combining baseline anti-GAD, anti-IAA, pancreatic volume, CD4+ central memory, CD4+ naïve, Th17 cells, CD8+ HLA-DR+CD38+, and NK CD56hi.