Autoantibody Development under Treatment with Immune-Checkpoint Inhibitors

Moel, E.C. de; Rozeman, E.A.; Kapiteijn, E.H.; Verdegaal, E.M.E.; Grummels, A.; Bakker, J.A.; ... ; Woude, D. van der

Article type: Cancer Immunology Miniature

Title: Autoantibody development under treatment with immune checkpoint inhibitors

Authors list: Emma C. de Moel M.D.[1], Elisa .A. Rozeman M.D.[2], Ellen H. Kapiteijn M.D. Ph.D.[4], Els M.E. Verdegaal Ph.D[4]., Annette Grummels B.Sc.[3], Jaap A. Bakker Ph.D. [3], Tom. W.J. Huizinga M.D. Ph.D.[1], John B. Haanen M.D. Ph.D.[2,4], René E.M. Toes Ph.D.[3], Diane van der Woude M.D. Ph.D.[1]

Affiliations:

[1] Department of Rheumatology, Leiden University Medical Center, Leiden, the Netherlands

[2] Netherlands Cancer Institute, Amsterdam, the Netherlands

[3] Department of Clinical Chemistry & Laboratory Medicine, Leiden University Medical Center, Leiden, the Netherlands

[4] Department of Medical Oncology, Leiden University Medical Center, Leiden, the Netherlands

Running Title: Autoantibodies and immune checkpoint inhibitors

Corresponding author:

Dr. Emma Corine de Moel

Department of Rheumatology

Leiden University Medical Centre

Albinusdreef 2, 2333ZA Leiden, the Netherlands

0031 (0)71 52 65652, e.c.de_moel@lumc.nl

The authors declare no potential conflicts of interest.

Funding: This project was funded by a ZonMw (the Netherlands Organisation for Health Research and Development) *Veni*-grant (91617100).

Word count full text: 2812 words

Word count without methods: 1720

Figures: 2

Tables: 1

Supplementary figures/tables: 4

Key words: autoantibodies, immune checkpoint inhibitors, ipilimumab, immune-related adverse events, melanoma

Abstract

Immune checkpoint inhibitors (ICIs) activate the immune system to assault cancer cells in a manner that is not antigen specific. We hypothesized that tolerance may also be broken to autoantigens, resulting in autoantibody formation, which could be associated with immune-related adverse events (irAEs) and antitumor efficacy. Twenty-three common clinical autoantibodies in pre- and post-treatment sera from 133 ipilimumab-treated melanoma patients were determined, and their development linked to the occurrence of irAEs, best overall response, and survival. Autoantibodies developed in 19.2% (19/99) of patients who were autoantibody-negative pre-treatment. A non-significant association was observed between development of any autoantibodies and any irAEs (OR: 2.92 [95% CI: 0.85 to 10.01]). Patients with anti-thyroid antibodies after ipilimumab had significantly more thyroid dysfunction under subsequent anti–PD-1 therapy: 7/11 (54.6%) patients with anti-thyroid antibodies after ipilimumab developed thyroid dysfunction under anti–PD1 versus 7/49 (14.3%) patients without antibodies (OR: 9.96 [95% CI: 1.94 to 51.1]). Patients who developed autoantibodies showed a trend for better survival (HR for all-cause death: 0.66 [95% CI: 0.34 to 1.26]) and therapy response (OR: 2.64 [95% CI: 0.85 to 8.16]). We conclude that autoantibodies develop under ipilimumab treatment and could be a potential marker of ICI toxicity and efficacy.

Introduction

Immune checkpoint inhibitors (ICIs) have improved the previously dismal prognosis of patients with various types of cancer, but at the cost of immune-related adverse events (irAEs) including arthritis, colitis, hepatitis, and various endocrinopathies [1]. ICIs inhibit negative costimulatory signals to T cells, thereby enhancing antitumor T-cell responses [2]. Because this mode of action is not antigen-specific, ICIs may also (re)activate otherwise dormant autoreactive T-cells. This in turn might lead to a break in T-cell tolerance to not only tumor antigens but also autoantigens, resulting in activation of autoreactive B-cells and ultimately the formation of autoantibodies. If true, the occurrence of autoantibodies may be associated with more frequent irAEs. Production of autoantibodies may indicate enhanced global immunogenicity, which may, in turn, be associated with better antitumor responses, as has been reported for changes in the T-cell repertoire [3-5]. Therefore, we determined if autoimmune disease-associated autoantibodies were formed with ICI treatment and investigated their association with irAEs and clinical outcome.

Methods

Patients and serological measurements

For this analysis, we included one hundred thirty-three patients with late-stage melanoma who were treated with ipilimumab, a CTLA-4-inhibitor, for whom pre- and post-treatment serum or plasma samples were available. Patients were treated with a maximum of four cycles ipilimumab 3mg/kg in an expanded access program or according to the label after approval at the Netherlands Cancer Institute or the Leiden University Medical Center. Patients were included if they were at least 18 years of age and had histologically or cytologically proven irresectable stage IIIc or IV melanoma with measurable metastatic

lesions according to the RECIST 1.1 criteria. Patients were treated with four cycles of intravenous 3 mg/kg ipilimumab every 3 weeks. Sixty-six (49.6%) of patients were treated with anti-PD1 therapy following ipilimumab: either 2 mg/kg intravenous pembrolizumab every three weeks, or 240 mg intravenous nivolumab every 4 weeks. The study was conducted in accordance with the Declaration of Helsinki after approval by the institutional review boards of both centers. All patients signed informed consent for withdrawal of extra blood samples for biomarker analysis. According to the study protocol, serum or plasma for autoantibody determination was collected before initiation of ipilimumab treatment and 12 weeks after. Pre- and post-treatment serum was snap-frozen and stored at -80 degrees until autoantibody determination. Due to failed measurements, post-ipilimumab autoantibody status could not be determined in 4 patients (Supplementary Fig. S1).

Indirect immunofluorescence assays

Autoimmune hepatitis and primary biliary cirrhosis-associated anti-smooth muscle, anti-mitochondrial, and anti-liver/kidney microsome (LKM) antibodies were measured by indirect immunofluorescence assay (IFA) using mouse liver/kidney/stomach substrate (Aesku). Anti-nuclear antibodies (ANA) were determined in all patients by IFA using the HEp-2000™ ANA Test System which uses human epithelioid cells stably transfected with the SSA/Ro autoantigen, cultured and fixed directly on the test wells (Immuno Concepts). Patient serum samples at a dilution of 1:40 were incubated with antigen substrate for 30 minutes at room temperature to allow specific binding of autoantibodies to cell nuclei. After washing with phosphate-buffered saline to remove non-specifically bound antibodies, the substrate was incubated with an anti-human antibody conjugated to fluorescein. After another washing step, the nuclear staining pattern was read using the international consensus on antinuclear antibody pattern (ICAP; [6]) by two experienced, independent readers trained in ANA-

pattern reporting and blinded to time-order and patient data of samples. In the case of lack of consensus, a third reader functioned as tie-breaker. All system reagents, conjugates, calibrators, and positive and negative controls were provided by and used according to instructions of the manufacturer (Immuno Concepts). All steps of the IFA were conducted using a Helmed fully automated IFA slide processor (Aesku).

Fluorescence enzyme immunoassays

Anti-cyclic citrullinated peptide 2 (CCP2) IgG, rheumatoid factor (RF) IgM, anti-gliadin IgG, and (if ANA was positive by IFA) antibodies to extractable nuclear antigens (ENA) were determined by EliA™ technique on a Phadia™ ImmunoCap 250 instrument (Thermo Fisher Scientific). This is a fully automated and high-throughput fluorescence enzyme immunoassay system used for routine diagnostic laboratory testing. The fluorescence signal of measured serum samples is compared to calibrators with known concentrations. For anti-CCP2 IgG, citrullinated synthetic peptides (second generation antigen) were used as antigen, for RF IgM, aggregated rabbit IgG was used, for anti-gliadin IgG, synthetic deamidated gliadin peptides were used, and for ENA, a Symphony Well™ of various antigens was used: human recombinant U1RNP (RNP70, A, C), SS-A/Ro (60 kDa, 52 kDa), SS-B/La, Centromere B, Scl-70 protein, Jo-1 protein, and native purified Sm proteins. Anti-ENA positive patients were further assayed for the following specific ENA antibodies by EliA (coated antigens in parentheses): anti-SSA (human recombinant SS-A/Ro (60 kDa, 52 kDa) proteins), anti-SSB (human recombinant SS-B/La protein), anti-RNP70 (human recombinant RNP70 protein), anti-U1RNP (human recombinant U1RNP (RNP70, A, C) proteins), anti-Smith (synthetic SmD3 peptide), anti-Jo1 (human recombinant Jo-1 protein), anti-CENP (human recombinant centromere protein B), anti-PMSCL (human recombinant PM-Scl protein), anti-RNAP3 (human recombinant RNA polymerase III protein), anti-Scl70s (human recombinant Scl-70

protein). All system reagents, conjugates, calibrators, and positive and negative controls were used according to manufacturer's instructions.

Chemiluminescent immunoassays

Anti-thyroid peroxidase (TPO), anti-thyroglobulin (TG), and, in ANA-positive patients, anti-dsDNA were determined by non-competitive chemiluminescent immunoassay (CLIA) using Immulite 2000™ (Siemens Healthineers). These assays use a luminescent adamantyl dioxetane phosphate tracer and were performed using reagents provided by the manufacturer according to instructions in the package insert.

Clinical data

Information about demographics, treatment response, survival status, and the occurrence of irAEs was obtained from retrospective review of medical records. irAEs were recorded starting from the first ipilimumab treatment until one year later, death, or the start of different therapy (whichever occurred first), using Common Terminology Criteria for Adverse Events (CTCAE) version 4.03: any grade arthralgia/arthritis, colitis, hypophysitis, primary adrenal insufficiency, primary thyroid dysfunction, dermatitis (rash, vitiligo, or psoriasis), uveitis, or grade 3-4 hepatitis. Primary thyroid dysfunction as an irAE during anti–PD-1 treatment (nivolumab or pembrolizumab) following ipilimumab treatment was determined in the same manner. Hematological and serum parameters necessary for making the above diagnoses were determined at baseline, every 3 months during follow-up, at progressive disease, and according to the treating oncologist's clinical judgment. Three patients had pre-existing hypothyroidism. Thyroid dysfunction was only registered as an irAE in these patients if symptoms were aggravated and a new medical intervention was indicated.

Two of the four cases of arthralgia/arthritis constituted a flare of pre-existing rheumatoid arthritis (RA). Survival was defined as time from start of ipilimumab to death of any cause, recorded between start of first ipilimumab treatment until January 2018, for a median follow-up time of 20.4 months (IQR: 8.8-40.8). Radiologic evaluation (CT or PET/CT scanning) was performed at baseline, week 12, and subsequently every 3 months until progression. Response was scored according to RECIST 1.1 criteria. Best overall response was defined as the best response recorded from start of ipilimumab until date of progression, death, or the start of a different therapy (whichever occurred first). Patients achieving a partial response or complete response were considered responding patients.

Statistical analysis

We used McNemar's test for paired data to test whether autoantibody positivity increased post-ipilimumab. Frequencies of irAEs in patients who developed antibodies versus those who did not were compared using Fisher's exact tests. To test whether post-ipilimumab autoantibody positivity was associated with 1) the development of any irAEs under ipilimumab, primary thyroid dysfunction under subsequent anti–PD-1 therapy, or better overall response, and 2) overall survival, binary logistic regression and Cox proportional hazards regression, respectively, were used, and adjusted for age, gender, treating hospital, and number of ipilimumab cycles received. All analyses were conducted using Stata statistical software, Special Edition, release 14.1 (StataCorp LP).

Results

Autoantibody development

Mean age was 59 years (standard deviation: 14), and 62% of patients were male. Of 127 patients with complete pre-ipilimumab autoantibody data, 26 (20%) were positive for

any of the autoantibodies before treatment. In total, 29% (36/125) of patients with complete autoantibody data were autoantibody-positive after ipilimumab treatment. Of patients who were fully autoantibody-negative before ipilimumab treatment, 19.2% (19/99) developed any autoantibodies post-treatment ($p<0.0001$). Predominantly anti-TPO (4.8%, 6/125) and anti-TG (6.0%, 8/132) appeared in patients who were negative for these autoantibodies at baseline ($p=0.03$ and $p=0.008$, respectively). For all other autoantibodies, post-treatment positivity did not greatly change (Supplementary Fig. S1).

Association of autoantibodies with irAEs under ipilimumab

A non-significant association was seen between the development of any autoantibody and irAEs: 15/19 (78.9%) patients who developed any autoantibody experienced irAEs compared to 46/80 (57.5%) patients who did not develop autoantibodies (OR: 2.92 [95% CI: 0.85 to 10.01]) (Fig. 1). When disregarding autoantibody status pre-ipilimumab, patients with autoantibodies post-treatment also experienced more irAEs (Supplementary Fig. S2). No significant association between pre-ipilimumab autoantibody positivity and irAEs was observed: 8/26 (31%) patients who were autoantibody-positive pre-ipilimumab experienced irAEs compared to 38/100 (37%) patients who were autoantibody-negative pre-ipilimumab (OR: 1.61 [95% CI: 0.62 to 4.18]; $p=0.33$).

In a pre-specified subgroup analysis, we focused only on the irAEs related to the tested autoantibodies (arthritis/arthralgia, hepatitis, thyroid dysfunction, colitis, adrenal insufficiency, dermatitis, or sicca symptoms). In this analysis, 14/19 (73.7%) patients who developed autoantibodies had irAEs related to the tested antibodies compared to 37/80 (46.3%) patients who did not develop autoantibodies, indicating a significant association between the development of autoantibodies and irAEs (OR: 3.64 [95% CI: 1.13 to 11.75]). However, the appearance of a specific autoantibody did not associate with the occurrence of

an irAE in the organ system affected by the disease for which the specific autoantibody has diagnostic value (Table 1).

Association of autoantibodies with thyroid dysfunction under anti-PD-1 therapy

We hypothesized that autoantibody development with ipilimumab treatment might predispose patients to irAEs during subsequent anti–PD-1 therapy. Following progression on ipilimumab treatment and after exclusion of patients who had thyroid dysfunction with ipilimumab (n=12), 61 (50.4%) patients received anti–PD-1 therapy. In these patients, we found a significant association between the development of thyroid autoantibodies while on ipilimumab and subsequent thyroid dysfunction under PD-1 blockade: 4/9 (44.4%) patients who developed thyroid autoantibodies with ipilimumab and subsequently received anti–PD1-therapy had thyroid dysfunction under anti–PD-1 compared to only 7/48 (14.6%) patients who did not develop autoantibodies (OR: 6.26 [95% CI: 1.07 to 36.5]; p=0.04). The association between the development of thyroid autoantibodies while on ipilimumab and subsequent thyroid dysfunction under PD-1 blockade was even stronger when autoantibody status pre-ipilimumab was disregarded and all anti–PD-1-treated patients were included in the analysis (n=60; one patient missing anti-TPO measurement): 7/11 (54.6%) patients who had thyroid autoantibodies after ipilimumab treatment developed thyroid dysfunction under anti–PD-1 compared to 7/49 (14.3%) patients who did not develop autoantibodies (OR: 9.96 [95% CI: 1.94 to 51.1]; p=0.006).

Association of autoantibody development with survival and response

We next investigated the association of autoantibody development following the initial ipilimumab treatment with survival and response. During the median follow-up time of 20.4 months (IQR: 8.8-40.8), 92 (69%) patients succumbed to disease after a median of 11.2 months (IQR 7.3-21.9), 87 patients (65%) had stable or progressive disease, and 46 patients

(35%) achieved complete or partial response. Patients who developed autoantibodies had a minor survival benefit compared to those that stayed autoantibody-negative, although this was not significant (HR for all-cause death: 0.66 [95% CI: 0.34 to 1.26]; p=0.21; Fig. 2). There was no significant association between the presence of a specific autoantibody and survival (Supplementary Table S1).

There was also a trend towards an association between development of any autoantibody and treatment responses (OR for response: 2.64 [95% CI: 0.85 to 8.16]; p=0.09). When assessing specific autoantibodies, only the development of thyroid autoantibodies was significantly associated with treatment response (OR for response: 5.43 [95% CI: 1.38 to 21.4]; p=0.02).

To determine whether the observed associations between autoantibody development and clinical outcomes were due to an association of irAEs with both entities, we also investigated the association between irAEs and clinical outcomes. No association between occurrence of irAEs and survival or treatment response was found (HR for all-cause death: 1.12 [95% CI: 0.70 to 1.79], p=0.64; OR for response: 1.53 [95% CI: 0.68 to 3.46], p=0.31). The estimates of the association between autoantibodies and survival and treatment response reported above did not greatly change after adjusting the analyses for occurrence of irAEs: HR for all-cause death: 0.65 [95% CI: 0.34 to 1.25], p=0.20; OR for response: 2.50 [95% CI: 0.80 to 7.84], p=0.12. All associations of autoantibody status with survival and treatment response were similar when autoantibody status pre-ipilimumab was disregarded and all patients were included in the analysis (Supplementary Table S2). Patients who were autoantibody-positive pre-ipilimumab had no survival or response benefit compared to patients who were autoantibody-negative (HR for all-cause death: 1.16 [95% CI: 0.67 to 2.03], p=0.59; OR for response: 0.53 [95% CI: 0.19 to 1.46]; p=0.22).

Discussion

In this study, we found that ipilimumab treatment induced development of autoantibodies in a fifth of melanoma patients. Our analyses revealed a trend for association between autoantibodies and irAEs under ipilimumab, and a much stronger, significant association between ipilimumab-induced thyroid autoantibodies and thyroid dysfunction under subsequent PD-1 blockade. Lastly, we found a minor survival and response benefit in patients who developed autoantibodies, specifically in those who developed thyroid autoantibodies.

We determined the presence autoantibodies both pre- and post-treatment with ICI therapy and linked these data to irAEs and clinical outcomes. Our results expand previous findings regarding the presence thyroid autoantibodies in patients with ICI-induced thyroid dysfunction [7-10] by showing that these autoantibodies also develop in the absence of overt thyroid dysfunction. Anti-thyroid antibodies are surprisingly common in populations without overt thyroid disease, associated with or induced by concomitant autoimmune disease (i.e.: type 1 diabetes mellitus, rheumatoid arthritis (RA), and Celiac's disease) [11-17], mutations in CTLA-4 [18, 19], upregulation of MHC class II molecules on thyrocytes leading to thyroid antigen presentation to autoreactive T-cells [20], or as we show here, ipilimumab treatment. Our data also confirm previous studies reporting that patients rarely develop RA autoantibodies [21-24] or autoimmune hepatitis antibodies even in the presence of the related irAE [25-28].

Our findings demonstrated that the development of thyroid autoantibodies predisposes euthyroid ipilimumab-treated patients to subsequent thyroid dysfunction under anti–PD-1 therapy. The association between thyroid autoantibodies and thyroid dysfunction under anti–PD-1 therapy has been described previously [29, 30]. Our results confirm that it is clinically

useful to monitor patients with pre-existing thyroid autoantibodies closely for thyroid dysfunction with anti–PD-1 therapy.

Although several studies report an association between irAEs and clinical outcome under ICIs [31, 32], we did not find such an association in this study. A relationship between immune-related thyroid dysfunction and clinical outcome has been described previously for various types of cancer immunotherapy, including IL2 [33-35], interferon-2α [35-37], and pembrolizumab [29]. Some of these studies also found a response or survival benefit under IL2 [33, 34] or interferon-2α [36] for patients who developed thyroid autoantibodies. These findings are in line with our observations that patients developing thyroid autoantibodies with ICI have a better treatment response.

Our results indicate that CTLA-4 inhibition may lead to loss of B-cell self-tolerance. ICIs execute their function in an antigen-independent manner by diversifying the T-cell repertoire against a multitude of tumor antigens [3, 5]. In this study, we found that tolerance to non-tumor autoantigens is broken as well and that breaking of tolerance may be associated with signs of clinical autoimmunity, under CTLA-4 inhibition as well as subsequent PD-1 blockade. We did not observe that specific autoantibodies induced disease in their related organ systems, but our results lacked the power to test this hypothesis. Breaking of B-cell tolerance and development of autoantibodies was also associated with better treatment response (statistical trend) and a survival benefit (though non-significantly). Previous studies have shown that greater expansion of the T-cell repertoire by ICIs is associated with better response [3, 5, 38]. We hypothesize that this expansion is paired with T cell–dependent activation of autoreactive B-cells and autoantibody production. If this is the case, autoantibodies may function as a marker for effective ICI-induced immunogenicity, and it is this enhanced immunity (rather than the autoantibodies themselves) which in turn leads to reactions against both clinically favorable (e.g.: tumor) and unfavorable (e.g.: non-

tumor/self) tissues. This could explain the link found in this study between autoantibodies and both treatment response and irAEs.

The main limitation of this study is a lack of power due to the limited number of patients. This may explain why some of our findings failed to reach statistical significance. We also had limited clinical data for which to correct our analyses. However, our study tested all patients treated with ipilimumab (not just the subset that developed irAEs) for a broad panel of autoantibodies in a longitudinal manner, facilitating a pre- and post-treatment comparison of autoantibody prevalence.

We conclude that the development of autoantibodies is common with ipilimumab treatment and that autoantibody presence is associated with the development of irAEs and a trend for better overall response and survival. These results indicate a promising avenue for future research in the quest for biomarkers predicting ICI therapy toxicity and efficacy.

Tables and Figure Legends

Table 1: Association between autoantibody development and irAEs.

	Converted to positive for…	**Stayed negative for…**	**p**
Any irAE / any antibody	15/19 (78.9%)	46/80 (57.5%)	0.12
Any autoantibody-related irAE † / any antibody	14/19 (73.7%)	37/80 (46.3%)	**0.04**
Arthralgia or arthritis / anti-CCP2 or RF	0/3 (0%)	3/121 (2.5%)	1.00
Hepatitis / autoimmune hepatitis antibodies‡	1/8 (12.5%)	4/109 (3.7%)	0.30
Thyroiditis / anti-TPO or anti-TG	2/13 (15.4%)	8/111 (7.2%)	0.28
Colitis / anti-endomysium or anti-gliadin IgG	0/2 (0%)	30/129 (23.3%)	1.00
Adrenal insufficiency / anti-adrenal cortex	0/0 (0%)	0/133 (0%)	N/A
Dermatitis / anti-nuclear antibodies	1/4 (25%)	33/122 (27%)	1.00
Sicca symptoms / anti-nuclear antibodies	0/4 (0%)	1/122 (0.8%)	1.00

In each cell, n/N indicates the number of patients who developed the irAE (n) out of the total number who converted to positive or stayed negative for the indicated antibody (N). p-values are calculated by Fisher's exact test. † arthritis/arthralgia, hepatitis, thyroid dysfunction, colitis, adrenal insufficiency, dermatitis, or sicca symptoms. ‡ anti-smooth muscle, anti-mitochondria, anti-liver-kidney-microsome, or anti-nuclear antibodies.

Figure Legends

Figure 1: Frequency of irAEs in pre-ipilimumab autoantibody-negative patients who did not develop autoantibodies (left) versus those who developed autoantibodies (right) after ipilimumab treatment.

Figure 2: Overall survival in patients who were negative for autoantibody at baseline. Patients who developed autoantibodies (n=19) were compared to those who did not develop autoantibodies (n=80). Numbers below the graph indicate the number of patients at risk within each group.

References

1. Bertrand A, Kostine M, Barnetche T, Truchetet ME, Schaeverbeke T. Immune related adverse events associated with anti-CTLA-4 antibodies: systematic review and meta-analysis. BMC Med. 2015;13:211.

2. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252-64.

3. Kvistborg P, Philips D, Kelderman S, Hageman L, Ottensmeier C, Joseph-Pietras D, et al. Anti-CTLA-4 therapy broadens the melanoma-reactive CD8+ T cell response. Sci Transl Med. 2014;6:254ra128.

4. Oh DY, Cham J, Zhang L, Fong G, Kwek SS, Klinger M, 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;77:1322-30.

5. Robert L, Tsoi J, Wang X, Emerson R, Homet B, Chodon T, et al. CTLA4 blockade broadens the peripheral T-cell receptor repertoire. Clin Cancer Res. 2014;20:2424-32.

6. Chan EK, Damoiseaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francescantonio PL, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. Front Immunol. 2015;6:412.

7. Guaraldi F, La Selva R, Sama MT, D'Angelo V, Gori D, Fava P, et al. Characterization and implications of thyroid dysfunction induced by immune checkpoint inhibitors in real-life clinical practice: a long-term prospective study from a referral institution. J Endocrinol Invest. 2017.

8. Morganstein DL, Lai Z, Spain L, Diem S, Levine D, Mace C, et al. Thyroid abnormalities following the use of cytotoxic T-lymphocyte antigen-4 and programmed death

receptor protein-1 inhibitors in the treatment of melanoma. Clin Endocrinol (Oxf). 2017;86:614-20.

9. Sznol M, Postow MA, Davies MJ, Pavlick AC, Plimack ER, Shaheen M, et al. Endocrine-related adverse events associated with immune checkpoint blockade and expert insights on their management. Cancer Treat Rev. 2017;58:70-6.

10. Villa NM, Farahmand A, Du L, Yeh MW, Smooke-Praw S, Ribas A, et al. Endocrinopathies with use of cancer immunotherapies. Clin Endocrinol (Oxf). 2018;88:327-32.

11. Cardenas Roldan J, Amaya-Amaya J, Castellanos-de la Hoz J, Giraldo-Villamil J, Montoya-Ortiz G, Cruz-Tapias P, et al. Autoimmune thyroid disease in rheumatoid arthritis: a global perspective. Arthritis. 2012;2012:864907.

12. Atzeni F, Doria A, Ghirardello A, Turiel M, Batticciotto A, Carrabba M, et al. Anti-thyroid antibodies and thyroid dysfunction in rheumatoid arthritis: prevalence and clinical value. Autoimmunity. 2008;41:111-5.

13. Yavasoglu I, Senturk T, Coskun A, Bolaman Z. Rheumatoid arthritis and anti-thyroid antibodies. Autoimmunity. 2009;42:168-9.

14. Przygodzka M, Filipowicz-Sosnowska A. Prevalence of thyroid diseases and antithyroid antibodies in women with rheumatoid arthritis. Pol Arch Med Wewn. 2009;119:39-43.

15. Grzelka A, Araszkiewicz A, Uruska A, Zozulinska-Ziolkiewicz D. Prevalence of anti-thyroid peroxidase in adults with type 1 diabetes participating in Poznan Prospective Study. Adv Clin Exp Med. 2015;24:79-84.

16. Sharifi F, Ghasemi L, Mousavinasab N. Thyroid function and anti-thyroid antibodies in Iranian patients with type 1 diabetes mellitus: influences of age and sex. Iran J Allergy Asthma Immunol. 2008;7:31-6.

17. Kalyoncu D, Urganci N. Antithyroid antibodies and thyroid function in pediatric patients with celiac disease. Int J Endocrinol. 2015;2015:276575.

18. Tomer Y, Greenberg DA, Barbesino G, Concepcion E, Davies TF. CTLA-4 and not CD28 is a susceptibility gene for thyroid autoantibody production. J Clin Endocrinol Metab. 2001;86:1687-93.

19. Zaletel K, Krhin B, Gaberscek S, Hojker S. Thyroid autoantibody production is influenced by exon 1 and promoter CTLA-4 polymorphisms in patients with Hashimoto's thyroiditis. Int J Immunogenet. 2006;33:87-91.

20. Bottazzo GF, Pujol-Borrell R, Hanafusa T, Feldmann M. Role of aberrant HLA-DR expression and antigen presentation in induction of endocrine autoimmunity. Lancet. 1983;2:1115-9.

21. Cappelli LC, Gutierrez AK, Baer AN, Albayda J, Manno RL, Haque U, et al. Inflammatory arthritis and sicca syndrome induced by nivolumab and ipilimumab. Ann Rheum Dis. 2017;76:43-50.

22. Kostine M, Rouxel L, Barnetche T, Veillon R, Martin F, Dutriaux C, et al. Rheumatic disorders associated with immune checkpoint inhibitors in patients with cancer-clinical aspects and relationship with tumour response: a single-centre prospective cohort study. Ann Rheum Dis. 2017.

23. Lidar M, Giat E, Garelick D, Horowitz Y, Amital H, Steinberg-Silman Y, et al. Rheumatic manifestations among cancer patients treated with immune checkpoint inhibitors. Autoimmun Rev. 2018.

24. Calabrese C, Kirchner E, Kontzias K, Velcheti V, Calabrese LH. Rheumatic immune-related adverse events of checkpoint therapy for cancer: case series of a new nosological entity. RMD Open. 2017;3:e000412.

404 25. Ahmed T, Pandey R, Shah B, Black J. Resolution of ipilimumab induced severe
405 hepatotoxicity with triple immunosuppressants therapy. BMJ Case Rep. 2015;2015.
406 26. Forschner A, Schraml C, Pierchalla K, Weide B, Eigentler TK, Lauer UM, et al.
407 Pembrolizumab-induced hepatitis: diagnosis and treatment. J Dtsch Dermatol Ges.
408 2017;15:933-5.
409 27. Johncilla M, Misdraji J, Pratt DS, Agoston AT, Lauwers GY, Srivastava A, et al.
410 Ipilimumab-associated Hepatitis: Clinicopathologic Characterization in a Series of 11 Cases.
411 Am J Surg Pathol. 2015;39:1075-84.
412 28. Spankuch I, Gassenmaier M, Tampouri I, Noor S, Forschner A, Garbe C, et al. Severe
413 hepatitis under combined immunotherapy: Resolution under corticosteroids plus anti-
414 thymocyte immunoglobulins. Eur J Cancer. 2017;81:203-5.
415 29. Osorio JC, Ni A, Chaft JE, Pollina R, Kasler MK, Stephens D, et al. Antibody-
416 mediated thyroid dysfunction during T-cell checkpoint blockade in patients with non-small-
417 cell lung cancer. Ann Oncol. 2017;28:583-9.
418 30. Kobayashi T, Iwama S, Yasuda Y, Okada N, Tsunekawa T, Onoue T, et al. Patients
419 With Antithyroid Antibodies Are Prone To Develop Destructive Thyroiditis by Nivolumab:
420 A Prospective Study. J Endocr Soc. 2018;2:241-51.
421 31. Sznol M, Ferrucci PF, Hogg D, Atkins MB, Wolter P, Guidoboni M, et al. Pooled
422 Analysis Safety Profile of Nivolumab and Ipilimumab Combination Therapy in Patients With
423 Advanced Melanoma. J Clin Oncol. 2017;35:3815-22.
424 32. Weber JS, Hodi FS, Wolchok JD, Topalian SL, Schadendorf D, Larkin J, et al. Safety
425 Profile of Nivolumab Monotherapy: A Pooled Analysis of Patients With Advanced
426 Melanoma. J Clin Oncol. 2017;35:785-92.

33. Atkins MB, Mier JW, Parkinson DR, Gould JA, Berkman EM, Kaplan MM. Hypothyroidism after treatment with interleukin-2 and lymphokine-activated killer cells. N Engl J Med. 1988;318:1557-63.

34. Franzke A, Peest D, Probst-Kepper M, Buer J, Kirchner GI, Brabant G, et al. Autoimmunity resulting from cytokine treatment predicts long-term survival in patients with metastatic renal cell cancer. J Clin Oncol. 1999;17:529-33.

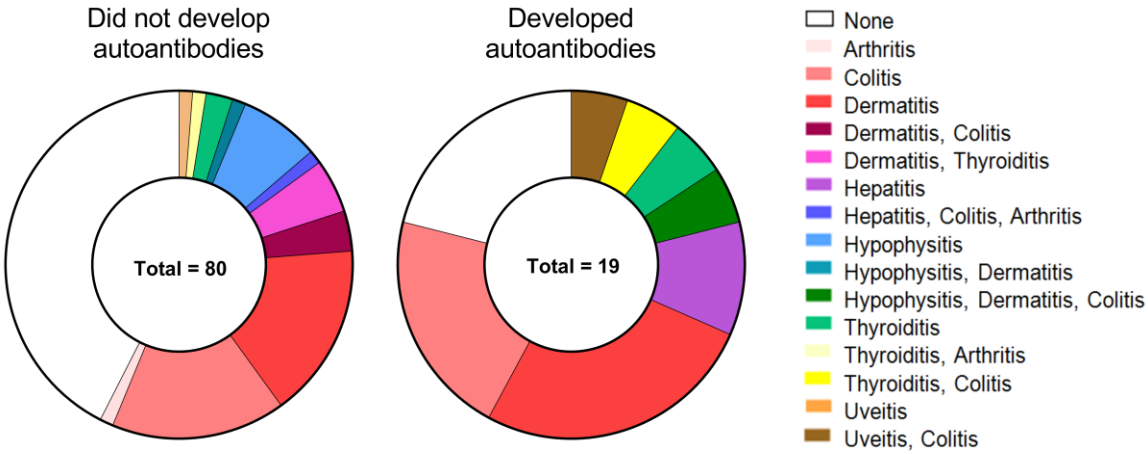
35. Reid I, Sharpe I, McDevitt J, Maxwell W, Emmons R, Tanner WA, et al. Thyroid dysfunction can predict response to immunotherapy with interleukin-2 and interferon-2 alpha. Br J Cancer. 1991;64:915-8.

36. Gogas H, Ioannovich J, Dafni U, Stavropoulou-Giokas C, Frangia K, Tsoutsos D, et al. Prognostic significance of autoimmunity during treatment of melanoma with interferon. N Engl J Med. 2006;354:709-18.

37. Bouwhuis MG, Suciu S, Collette S, Aamdal S, Kruit WH, Bastholt L, et al. Autoimmune antibodies and recurrence-free interval in melanoma patients treated with adjuvant interferon. J Natl Cancer Inst. 2009;101:869-77.

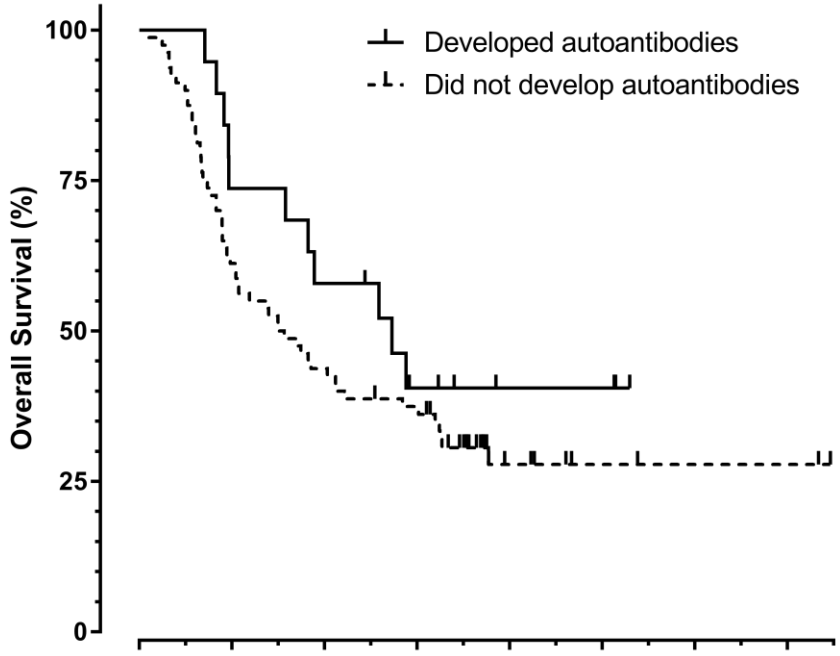
38. Oh DY, Cham J, Zhang L, Fong G, Klinger M, Faham M, et al. Association between T cell repertoire diversification and both clinical response as well as toxicity following immune checkpoint blockade in metastatic cancer patients. Journal of Clinical Oncology. 2016;34:3029-.

Figure 1



449

Figure 2



Months	0	12	24	36	48	60	72	84
Developed auto-antibodies, # at risk	19	14	11	6	3	3	0	0
Did not develop auto-antibodies, # at risk	80	49	35	29	8	4	3	3

450

451

Supplementary Figure S1: Heatmap of antibody positivity pre- and post-ipilimumab treatment. Not shown: all patients were anti-ENA negative at baseline, while at follow-up, two patients became anti-ENA positive, specifically anti-SSA positive.